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ARCHIVES OF DISEASE IN CHILDHOOD

The Journal of the British Paediatric Association

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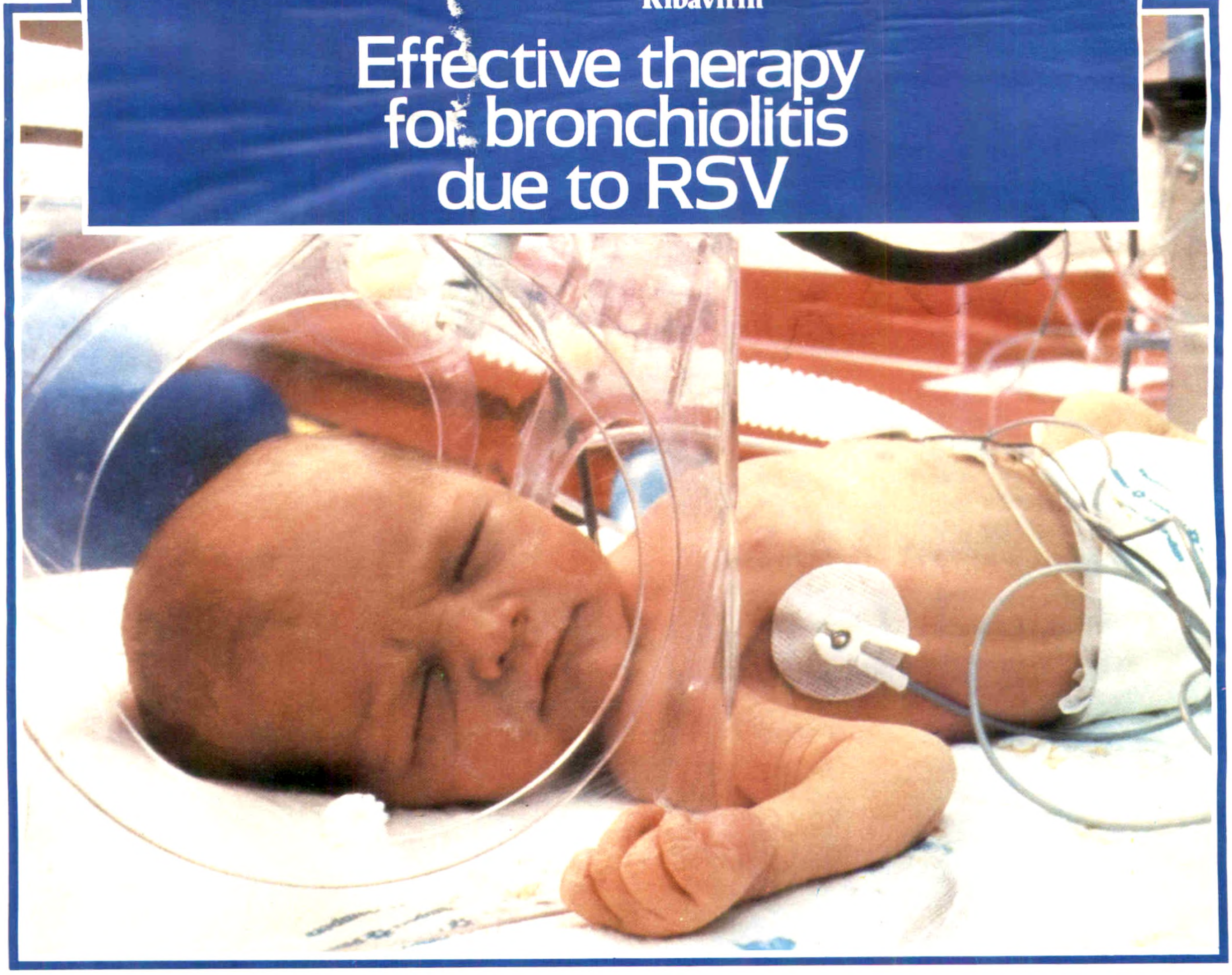

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Annotation

Antibiotic treatment of suspected neonatal meningitis

Neonatal meningitis remains a life threatening emergency with a mortality of 20–50%; 50% of the survivors sustain major sequelae.[1 2] Fortunately the condition is uncommon, the incidence being about 0·4 to 1 per thousand live births.[3] Early diagnosis and treatment are vital if these figures are to improve. The commonest organisms are still group B streptococcus and *Escherichia coli*, although the range is wide.[4] *Listeria monocytogenes* is now the third most common cause in the United Kingdom. *Staphylococcus epidermidis* is an important pathogen after invasive neurosurgical procedures.

Choice of antibiotic

Meningitis is more often suspected than confirmed because the signs are usually subtle and non-specific. Lumbar punctures are therefore often performed as part of a septic screen. If the infant is very ill at the time and probably bacteraemic there is a risk of seeding organisms into the meninges. In this situtation treatment should include an antibiotic that is known to penetrate the cerebrospinal fluid. Our current recommendation is cefotaxime (100 mg/kg/24 hours divided into two doses), penicillin (100 mg/kg/24 hours divided into two doses), and gentamicin (2·5 mg/kg every 12 hours or every 18 hours for infants less than 1000 g with adjustment according to drug concentrations) with early rationalisation of treatment once the results of microscopy of the cerebrospinal fluid and culture are known. Listeria will not respond to cephalosporins, therefore cover with this agent alone will be inadequate. Chloramphenicol (50 mg/kg/24 hours in two divided doses) is now not a good choice[5 6]; the results are unimpressive, the therapeutic margin is narrow, and organisms readily become resistant. Furthermore, the drug is not bactericidal against the anaerobic Gram negative bacilli and can interfere with the bactericidal effect of gentamicin.[7] It is subject to dosage and dilution errors and cannot be dialysed out rapidly by the peritoneal route if an overdose has been given. Chloramphenicol is metabolised to its inactive metabolites by microsomal glucuronyl transferase. This enzyme system is immature in the newborn, and is the route for metabolism of other drugs including the anticonvulsants phenobarbitone and phenytoin, which are often required in babies with meningitis. If chloramphenicol is used access to a rapid drug assay is needed and this is rarely available.

E coli and other Gram negative organisms

These still present major problems despite the advent of the cephalosporins. Although the cumulative experience with these agents in the neonate must be greater than the 26/240 patients reported in 12 trials,[8] mortality has not improved as much as one would have hoped. Cefotaxime is currently the most effective cephalosporin; there have been treatment failures with cefuroxime, which is not always effective against Gram negative organisms, and ceftazidime should be reserved for pseudomonas infections. Gentamicin should be continued primarily to treat the associated septicaemia.

Cerebral ultrasound should always be performed as soon as possible. If the ventricles are enlarged with a ventricular index measurement above the 97th centile[9] there is no doubt in our minds that intraventricular treatment should be used. Intrathecal treatment using the lumbar route of administration is ineffective, probably due to a failure of drug to reach the ventricular cerebrospinal fluid. Intraventricular treatment should be administered by a surgically implanted Rickham reservoir with daily instillation of 1 mg of gentamicin for seven days. The system allows measurement and control of cerebrospinal fluid pressure, together with bacteriological monitoring and avoids the problems associated with the development of porencephalic cysts along the needle tracks of repeated ventricular punctures. In a trial involving 61 centres in the United States and South America, McCracken and his colleagues reported the outcome of 52 infants randomised to receive systemic treatment plus intraventricular treatment compared with systemic treatment alone.[10] The study was stopped early because of a higher mortality in the group given intraventricular treatment, but many of the infants had salmonella infection and were treated relatively late. There has been no study of intraventricular treatment using treatment by a reservoir with concomitant systemic administration of cephalosporins and little information on the ventricular drug concentration achieved after systemic treatment alone in dilated ventricles.

When the ventricles are normal in size we would do a diagnostic ventricular tap under ultrasound control if there was no clinical improvement at between 24 and 48 hours, taking the opportunity to instil a single dose of gentamicin and to measure the cerebrospinal fluid pressure. If the examination of the fluid confirmed that ventriculitis was present we would ask our neurosurgical colleagues to attempt to insert a Rickham reservoir in order to administer intraventricular antibiotics. In babies who were improving at 24–48 hours we would repeat the lumbar puncture and only perform a ventricular tap if organisms were still seen.

It is generally accepted that treatment should be continued for three weeks after sterilisation of the cerebrospinal fluid. Progress should be monitored by following the platelet count, white cell count, and C reactive protein concentration; there should be repeat lumbar puncture if any of these are failing to follow the expected improvement. Ventricular size should be monitored by ultrasound three times a week. A computed tomogram is occasionally necessary to diagnose subdural collections or abscess formation. Proteus has a particular prediliction for abscess formation,[11] therefore all patients in whom this organism is grown should have a computed tomogram.

The outlook for Gram negative meningitis remains grim, with a 20% mortality and with 38% of the survivors suffering major sequelae. We feel that an improvement can only be made if the suggested aggressive approach is adopted.

Organisms other than coliforms

Group B streptococcus still accounts for most cases of neonatal meningitis. The outlook is better than that for Gram negative infections and treatment should be for three weeks with penicillin and gentamicin. Dual treatment is advised because of the synergy between these two agents. Intraventricular treatment is seldom, if ever, necessary.

L monocytogenes is an emerging cause of meningitis in babies. Both ampicillin and penicillin are extremely effective in high dose, and either of these should be combined with gentamicin. The prognosis is better than for Gram negative meningitis.

A special problem arises in infants with ventriculoperitoneal shunts. These are prone to infection, with many units reporting a 30% infection rate after first insertion despite perioperative antistaphylococcal prophylaxis with flucloxacillin and vancomycin. *S epidermidis* is the usual culprit and the system needs to be exteriorised, after which both systemic antibiotics and intraventricular vancomycin treatment should be used. Caution is required before reinsertion of the system, ideally only when the cerebrospinal fluid has been sterile for at least two weeks.

Conclusion

Antibiotics alone do not treat meningitis, and the importance of intensive care cannot be overstressed. Most babies have an associated bacteraemia and many are shocked. Circulatory support using massive colloid/blood transfusion and inotropes, aggressive anticonvulsant treatment necessitating ventilation and the possible need for neurosurgical intervention mean that infants with Gram negative meningitis should be transferred to a unit with appropriate facilities and expertise. Those infected with other organisms may be cared for in units without intensive care facilities. Over the next decade there may be more information about the use of immunoglobulin and granulocyte transfusions in severe neonatal infections.

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ORIGINAL ARTICLES

Randomised trial of early tapping in neonatal posthaemorrhagic ventricular dilatation

Ventriculomegaly Trial Group

Abstract

Treatment of posthaemorrhagic ventricular dilatation by early repeated cerebrospinal fluid taps was compared with conservative management in a randomised controlled trial of 157 infants in 15 centres. Thirty infants died and six moved abroad before follow up. During the first 14 days after randomisation, the early treatment group had five times more taps, and 12 times more cerebrospinal fluid removed. Infection of the cerebrospinal fluid occurred in seven of the early treated and four of the conservatively managed infants. Of survivors, 62% in both groups ultimately had ventricular shunts.

Neurodevelopmental assessment of survivors at 12 months was carried out by a single experienced examiner. Of survivors, 103 (85%) had abnormal neuromotor signs and 88 (73%) had disabilities. There was no detectable benefit of early treatment for children who did not have parenchymal lesions at the time they entered the trial. Nearly all those with parenchymal lesions had neuromotor impairment, but early treatment was associated with a significant reduction in other impairments.

Progressive ventricular dilatation leading to hydrocephalus is a serious complication of cerebral intraventricular haemorrhage in preterm infants. More than half the survivors into childhood have cerebral palsy, and a third have global developmental delay.[1–4]

Posthaemorrhagic ventricular dilatation is thought to result from obstruction of the cerebrospinal fluid pathways, and arachnoid villi, by multiple small clots. Later, basal cistern arachnoiditis may produce permanent obstruction leading to hydrocephalus.[5] Ventricular size can be monitored easily by repeated real time ultrasound measurements of ventricular width, and centile values are available relating this to gestational age.[6]

Progressive ventricular dilatation and head enlargement is usually accompanied by some increase in cerebrospinal fluid pressure, although the upper limit of normal (0·80 kPa or 6 mm Hg) is only slightly exceeded in some affected infants.[7] It is not known to what extent additional neurological damage may result from a period of raised ventricular pressure. Increased cerebrospinal fluid pressure may cause periventricular oedema, distortion of developing pathways, and decreased cerebral perfusion; many of the affected infants may, however, have already sustained cerebral hypoxic-ischaemic damage in the perinatal period, which is likely to result in later impairments.

If cerebrospinal fluid pressure is raised, removal of some of the fluid over a number of days might improve the long term outcome. Impairment might be prevented, or, if hypoxic-ischaemic damage has already occurred, its extent might be limited to that caused by the pre-existing lesion. Observational studies[3 4 8–10] have suggested that serial removal of cerebrospinal fluid may improve long term outcome, but no benefit of such treatment in reducing the need for surgical shunting was detected in two small randomised controlled trials.[11 12] Dykes *et al* carried out a randomised trial of early treatment by lumbar puncture compared with close observation.[13] Severe posthaemorrhagic hydrocephalus was assessed by visual assessment of computed tomography or ultrasound scanning rather than by a quantitative method. Thirty eight asymptomatic infants were randomised of whom 31 survived. Among the 29 infants examined at 3–6 years of age there was a lower proportion of children with major handicap in the group that had received early treatment, but the small numbers involved did not reach statistical significance.

Repeated removal of cerebrospinal fluid may carry risks, such as infection and apnoea. Drainage by lumbar puncture, even if initially successful, may not be possible in some babies,[11 12] and ventricular taps may then be necessary to reduce pressure, thereby increasing the risks further. Small ill infants are not usually suitable for immediate permanent shunts and there is a high risk of shunt infection. A high concentration of protein in the cerebrospinal fluid is an additional reason for delaying shunt surgery, because it predisposes to blockage of the shunt.[14]

Because of the uncertainties about the value of early treatment, we mounted a large, multicentre randomised trial in which early mangement of posthaemorrhagic ventricular dilatation by repeated cerebrospinal fluid taps was compared with conservative management. The principal outcome measure was the long term neurodevelopmental condition in the survivors. We report here the results of the trial up to the (corrected) age of 12 months.

Patients and methods

Infants with posthaemorrhagic ventricular dilatation were recruited from 15 centres during the three years, 1984–7. The final protocol was agreed by one neonatologist responsible for the trial in each of the participating centres and

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then approved by each local research ethics committee. In some centres, signed parental consent was required; in others a verbal explanation was considered more appropriate.

ELIGIBILITY

Infants were eligible if all the following criteria were met: (*i*) there was haemorrhage into the ventricles identified by ultrasound scan; (*ii*) the ventricular width (midline to lateral border of the ventricle) was below the 97th centile[6] on the first scan, or the first scan showed clot distending the ventricle above the 97th centile (the measurement was taken from the midline to the lateral border of the lateral ventricle at the level of the interventricular foramina on a coronal scan); (*iii*) serial measurements of ventricular width had shown a progressive increase; and (*iv*) the most recent measurement of ventricular width had reached 4 mm over the 97th centile. In the case of asymmetrical dilatation, the measurement of the smaller ventricle was taken.

These criteria were based on the experience of members of the group who suggested that about half the infants meeting these criteria would eventually require surgical shunts.

TREATMENT ALLOCATION

After criteria of eligibility had been confirmed, babies were entered into the trial by telephoning the Clinical Trial Service Unit in Oxford. The clinician first gave details of identification and was then told the treatment allocation. Random allocation to either early treatment with taps, or conservative management, was organised in balanced blocks of six, stratified by centre.

TREATMENT SCHEDULES

Early treatment

The object of early treatment was to prevent further ventricular dilatation. A lumbar puncture was carried out and cerebrospinal fluid allowed to drain until a maximum of 2% of the body weight had been drained. The baby was then observed carefully, with heart rate and respiration being monitored. Lumbar punctures were repeated as often as necessary if ventricular width increased by 2 mm or more above the measurement before the first tap. If lumbar puncture yielded no more than 2 ml cerebrospinal fluid, ventricular puncture could be performed. This was carried out through the anterior fontanelle at least 1 cm lateral to the midline, entering the larger lateral ventricle. If repeated taps were necessary for more than four weeks, and head enlargement continued, permanent shunting was discussed, but the decision to insert a shunt also depended on the general condition of the infant, the weight, and the protein concentration in the cerebrospinal fluid.

Conservative management

The appearance on ultrasound scans did not dictate treatment in the group that was managed conservatively. The criteria for removal of cerebrospinal fluid were: (*i*) excessive head enlargement for at least two weeks; namely, an increase in head circumference of twice the normal velocity for gestational age after entry to the trial[15]; or (*ii*) symptomatic raised intracranial pressure with a measured cerebrospinal fluid pressure greater than 1·60 kPa (12 mm Hg) (if a bulging fontanelle and neurological abnormality suggested raised intracranial pressure, a lumbar puncture or ventricular tap could be carried out to measure pressure).

The criteria for permanent shunting were the same in both treatment groups; that is, failure to control head size with no contraindication to shunting—for example, cardiopulmonary disease, infection, or a high concentration of protein in the cerebrospinal fluid.

MONITORING AND COMPLIANCE

In each neonatal unit one neonatologist was responsible for cranial ultrasound scanning (in a few hospitals in conjunction with a radiologist). Before infants were entered into the trial, three meetings of collaborators were held to reach agreement on scanning techniques, measurement of ventricles, and recognition of intraventricular haemorrhage and parenchymal cerebral lesions. A parenchymal lesion was judged to be present if there was persistent echodensity in the cerebral substance viewed in two planes. 'Parenchymal lesion' included both parenchymal 'extension' of periventricular haemorrhage and periventricular leucomalacia. Participants were specifically asked to document the presence or absence of any parenchymal lesions at the time of entry to the trial.

Scanning was usually carried out daily during the first week of life for infants having intensive care, and then weekly while in hospital. After entry to the trial, scanning was performed daily on most infants having early treatment with taps, as the decision to tap depended on whether there had been further ventricular enlargement. In the group managed conservatively scanning did not determine management but was, initially, carried out at least once every three days.

After entry into the trial, recordings were made of head circumference, ventricular width, lumbar and ventricular taps (and volume of cerebrospinal fluid removed), cerebrospinal fluid pressure on the day of entry and subsequently (if it had been measured), and details about the analysis of the cerebrospinal fluid (such as presence of organisms or cells and the protein concentration). Non-communicating post-haemorrhagic ventricular dilatation was defined as the inability to drain more than 2 ml of cerebrospinal fluid by lumbar puncture. The clinical coordinator could be contacted by telephone to clarify difficulties in carrying out the protocol, and he also monitored compliance with the protocol as case records were returned.

STATISTICAL CONSIDERATIONS

Before starting the trial it was estimated that 60 surviving infants in each treatment group would give an 80% chance of detecting a significant ($p<0·05$) reduction in severe impairments from

50% to 25%. We expected posthaemorrhagic ventricular dilatation to occur in 3–4% of infants of very low birth weight (<1500 g), and to be associated with a death rate of about 20%. The 15 neonatal intensive care units that collaborated admit roughly 1500 very low birthweight infants each year, equivalent to about 45–60 cases of posthaemorrhagic ventricular dilatation annually, so a recruitment period of three years was planned. Analyses have been on the groups as allocated, regardless of subsequent management. There were no interim analyses. Secondary analyses were stratified by whether a parenchymal lesion was recorded on entry to the trial. These investigated, firstly, whether early treatment reduces neuromotor impairment in children without a parenchymal lesion at the onset of treatment and, secondly, whether early treatment limits the extent of impairment other than neuromotor in those children with a pre-exisiting parenchymal lesion.

FOLLOW UP ASSESSMENT OF SURVIVORS AT 12 MONTHS CORRECTED AGE

Surviving children were assessed in their own homes at about one year after their expected date of delivery. All except three were seen by a single developmental paediatrician (Dr L Mutch) who was unaware of their original trial allocation; two Swiss children and one other child were assessed by local paediatricians. The assessment consisted of an interval history, a Griffiths's assessment of development,[16] and a standardised neurological examination as described by Amiel-Tison and Grenier.[17] Vision and hearing were assessed clinically to ensure that the child was able to participate in the developmental assessment, but full assessment of hearing and vision will not be available for all the children until a later age. Where the Griffiths's test was inappropriate because of severe visual deficit, a Reynell-Zinkin test was used to provide an approximate performance for age.[18]

For each child, outcome was summarised in two ways. Firstly, neuromotor signs were described in terms of their distribution and the severity of any motor impairment. Secondly, an overall assessment was made on the basis of all the findings including neurological signs, developmental condition, hearing and vision, health, and degree of alertness and interest, according to the scheme suggested by Amiel-Tison and Stewart.[19] Agreement was reached between the examiner and one of these authors on the classification of all the children based on the results of the examinations. The children were classed in three main groups as follows:

Normal

No neuromotor or neurosensory impairment was detected; Griffiths's developmental quotient was more than 70 and there were no neurobehavioural problems. For the purposes of overall assessment in this study, abnormal head size alone was not a criterion for assignment to an abnormal group.

Neuromotor impairment alone

Impairment was detected, but limited to the neuromotor system. This group was subdivided into those without disability, those with moderate disability, and those with severe disability.

Neuromotor plus other impairments

Multiple impairments were detected, affecting not only the neuromotor system. This group was subdivided into those with visual impairments, those with sensorineural hearing loss, those with developmental quotients of less than 70, and those with a combination of visual, hearing, and developmental impairments. All the children in this group were disabled. The aim of this scheme was to distinguish between impairments resulting from the initial event that preceded the ventricular dilatation, and subsequent impairment. Pre-existing injury is likely to be limited to the periventricular region and cause neuromotor impairment, albeit of variable severity. Raised intraventricular pressure could lead to damage beyond the periventricular region and cause other types of impairment.

Results

A total of 157 infants were enrolled in the trial during the three year period; 79 were allocated to have early treatment and 78 to be managed conservatively. During this period six infants in the collaborating centres who were known to have been eligible for enrolment were not entered, either because the parents did not consent or because the investigator was not available. Thirty infants died between entering the trial and the one year follow up, leaving 66 survivors in the early treatment group and 61 in the group managed conservatively. Three infants in each group could not be followed up because they had moved abroad.

Table 1 shows the characteristics of all the infants entering the trial, and of the 121 who were assessed at 12 months of age. Nearly all were of very low birth weight and there was a preponderance of boys. Randomisation achieved an acceptable balance of important variables between the two groups. Overall, roughly two thirds had parenchymal brain lesions and over 80% had ventriculospinal communication at the time of entry to the trial. There were fewer parenchymal lesions in the group allocated to early drainage of cerebrospinal fluid (59% compared with 71%). More children with parenchymal lesions subsequently died in the group managed conservatively, however, and the groups of survivors assessed at 12 months were similar in this respect (59% compared with 66%). The mean cerebrospinal fluid pressure at entry to the trial, roughly 1·20 kPa (9 mm Hg) in both groups, was three times the mean cerebrospinal fluid pressure in normal neonates, suggesting that most of the infants in this trial had ventricular enlargement caused by increased cerebrospinal fluid pressure.

Table 2 shows details of the early management of ventricular dilatation and confirms that there were pronounced differences in the treatment of the two groups during the early weeks.

Table 1 Description of the groups at entry to trial. Figures are mean (SD) except where otherwise stated

	All randomised		*Survivors seen at one year corrected age*	
	Early drainage of cerebrospinal fluid (n=79)	*Managed conservatively (n=78)*	*Early drainage of cerebrospinal fluid (n=63)*	*Managed conservatively (n=58)*
No (%) boys	57 (72)	49 (63)	45 (71)	36 (62)
Birth weight (g)	1220 (440)	1245 (436)	1239 (459)	1304 (478)
Gestational age at birth (weeks)	28·3 (2·8)	28·4 (2·9)	28·4 (3·0)	28·8 (3·3)
Age at trial entry (days)	19·1 (11·2)	15·9 (7·3)	19·7 (11·1)	15·6 (7·4)
No (%) with parenchymal brain lesion	47 (59)	55 (71)	37 (59)	38 (66)
No (%) not stated	3 (4)	1 (1)	2 (3)	1 (2)
Type of parenchymal lesion:				
No (%) bilateral	13 (16)	17 (22)	11 (17)	9 (16)
No (%) unilateral	29 (37)	36 (46)	24 (38)	28 (48)
No (%) not stated	5 (6)	2 (3)	2 (3)	1 (2)
Reason for eligibility:				
No (%) with progressive ventricular dilatation from <97th centile to 4 mm more than 97th centile for gestational age	70 (89)	68 (87)	56 (89)	51 (88)
No (%) with clots in ventricle with width >97th centile for gestational age on initial scan	6 (8)	10 (13)	4 (6)	7 (12)
No (%) not stated	3 (4)	0	3 (5)	0
No (%) with ventriculospinal communication	61 (77)	65 (83)	51 (81)	49 (84)
Pressure of cerebrospinal fluid (mm Hg)* (n=102)	9·2 (4·7)	8·9 (5·9)	9·1 (4·8)	8·3 (4·2)
Ventricular width (mm) (n=114)	17·4 (2·1)	17·8 (2·1)	17·4 (2·0)	18·0 (2·1)
Head circumference (cm) (n=122)	28·1 (2·8)	28·8 (3·3)	28·4 (2·9)	29·0 (3·1)

*1 kPa=7·5 mm Hg.

Table 2 Neonatal management of posthaemorrhagic ventricular dilatation by treatment allocation. Figures are given as number (%) except where otherwise stated

	All randomised		*Survivors seen at one year corrected age*	
	Early drainage of cerebrospinal fluid (n=79)	*Managed conservatively (n=78)*	*Early drainage of cerebrospinal fluid (n=63)*	*Managed conservatively (n=58)*
First 14 days (after day of entry)				
One or more taps	71 (90)	43 (55)	57 (90)	35 (60)
Median No of taps (10th and 90th centiles)	5 (0, 9)	1 (0, 5)	5 (1, 9)	1 (0, 4)
Management:				
Spinal taps only	39 (49)	27 (35)	31 (49)	21 (36)
Ventricular taps only	7 (9)	5 (6)	5 (8)	3 (5)
Spinal and ventricular taps	25 (32)	11 (14)	21 (33)	11 (19)
Median volume of cerebrospinal fluid removed (ml) (10th and 90th centiles)	58 (8, 158)	5 (0, 77)	61 (4, 161)	10 (0, 77)
Mean (SE) change in ventricular width (mm) (n=90)	0·3 (0·5)**	2·5 (0·6)**	0·0 (0·5)**	2·5 (0·6)**
Mean (SE) change in head circumference (cm) (n=122)	1·8 (0·2)*	2·4 (0·1)*	1·9 (0·3)*	2·5 (0·2)*
At 100 days (after day of entry)				
One or more taps	73 (92)	55 (71)	59 (94)	43 (74)
Median No of taps (10th and 90th centiles)	7 (1, 37)	4 (0, 28)	10 (1, 43)	4 (0, 31)
Management:				
Spinal taps only	33 (42)	22 (28)	25 (40)	16 (28)
Ventricular taps only	7 (9)	7 (9)	5 (8)	5 (9)
Spinal and ventricular taps or reservoir	33 (42)	26 (33)	29 (46)	22 (38)
Median volume of cerebrospinal fluid removed (ml) (10th and 90th centiles)	116 (14, 678)	38 (0, 556)	152 (10, 700)	53 (0, 556)
Other aspects				
Ventriculoperitoneal shunt insertion only	41 (52)	42 (54)	39 (62)	36 (62)
Choroid plexus coagulation	2 (3)	3 (4)	2 (3)	3 (5)
Died:				
Before hospital discharge	12 (15)	13 (17)	Not applicable	Not applicable
After discharge but before follow up assessment	1 (1)	4 (5)	Not applicable	Not applicable
Lost to follow up	3 (4)	3 (4)	Not applicable	Not applicable

*p=<0·5, **p=<0·01.

Table 3 Incidence of infection of the cerebrospinal fluid (meningitis or ventriculitis) in infants who had not (yet) had operations for hydrocephalus

Case No	*No of lumbar punctures before infection*	*No of ventricular taps before infection*	*Organism*	*Shunt subsequently carried out*	*Outcome*
Group receiving early treatment with taps					
1	11	6	Coagulase negative staphylococcus	Yes	Survived
2	0	3	Yeast*	No	Died
3	4	2	Yeast*	Yes	Survived
4	10	4	Coagulase negative staphylococcus	Yes	Survived
5	0	12	Not identified	No	Died
6	2	0	Yeast*	Yes	Survived
7	15	0	Yeast*	Yes	Survived
Group managed conservatively					
8	0	3	Coagulase negative staphylococcus	Yes	Survived
9	0	10	*Enterobacter* spp	Yes	Survived
10	2	0	Yeast*	No	Survived
11	7	1	*Klebsiella* spp	Yes	Survived

*Yeast=*Candida albicans* or *Hansenula anomala*.

Table 5 Assessment of development at follow up (Griffiths's scales). Figures are given as number (%)

	Early drainage of cerebrospinal fluid (n=63)	*Managed conservatively (n=58)*
Locomotor quotient:		
Not measureable	6 (10)	9 (16)
<50	6 (10)	10 (17)
50–69	17 (27)	16 (28)
70–99	19 (30)	11 (19)
≥100	15 (24)	22 (38)
Social scale quotient:		
Not measureable	6 (10)	9 (16)
<50	3 (5)	2 (3)
50–69	3 (5)	9 (16)
70–99	36 (57)	23 (40)
≥100	15 (24)	15 (26)
Hearing-speech quotient:		
Not measureable	6 (10)	9 (16)
<50	0	1 (2)
50–69	4 (6)	8 (14)
70–99	40 (63)	26 (45)
≥100	13 (21)	14 (24)
Eye-hand coordination quotient:		
Not measureable	6 (10)	9 (16)
<50	3 (5)	7 (12)
50–69	9 (14)	9 (16)
70–99	31 (49)	18 (31)
≥100	14 (22)	15 (26)
Performance quotient:		
Not measureable	6 (10)	9 (16)
<50	4 (6)	9 (16)
50–69	9 (14)	8 (14)
70–99	34 (54)	19 (33)
≥100	10 (16)	13 (22)
General quotient:		
Not measureable	6 (10)	9 (16)
<50	2 (3)	6 (10)
50–69	8 (13)	7 (12)
70–99	35 (56)	23 (40)
≥100	12 (19)	13 (22)

During the first 14 days after randomisation the early treatment group had a median of five cerebrospinal fluid taps compared with only one tap in the group managed conservatively (usually this was done to exclude meningitis), the volume of cerebrospinal fluid removed in the early treatment group was nearly 12 times greater than in the group treated conservatively, and this was associated with significantly smaller increases in ventricular width and head circumference.

By 100 days after entry many of the conservatively managed group had met the criteria for cerebrospinal fluid drainage, and the differences in management between the two treatment groups were therefore less pronounced. It was common for infants to have ventriculospinal communication at first, which later become non-communicating. Thus half the infants in the early treatment group eventually had one or more ventricular tap.

Infection of the cerebrospinal fluid was common even in infants who had not (yet) had any surgery for hydrocephalus (table 3). Seven infants in the early treatment group (9%) developed ventriculitis (without having had an operation) and two of these died. Four infants in the group managed conservatively (5%) developed ventriculitis (without having had an operation).

Overall, 30 children (19%) died during the first year, 13 (16%) in the early treatment group and 17 (22%) in the group managed conservatively. Of the survivors, 75 (62%) had ventricular shunts inserted, and six (5%) moved abroad and were lost to follow up; there were no differences between the trial groups (table 2). Nor were there clear differences in morbidity during the time between discharge from hospital and the follow up assessment (table 4); about 70% of both groups were readmitted to hospital, many more than once, and often for revision of their shunts.

Table 5 shows Griffiths's developmental scales one year after full term. Quotients could not be measured in 15 children because their vision was too severely impaired; all six such children in the early treatment group, and six of the nine in the group managed conservatively were judged to have developmental delay. Almost half of the survivors scored less than 70 on the Griffiths's locomotor scale, about a third scored less than 70 on the performance and eye-hand coordination scales, and about a quarter scored less than 70 on the personal-social, hearing-speech, and overall scales. Taking into account the developmental assessment of the 15 'blind' children, 16 children in the early treatment group (25%) and 19 children in the group managed conservatively (33%) had developmental delay (relative risk 0·78; 95% confidence interval (CI) 0·44 to 1·36).

Table 4 Morbidity between discharge from hospital and follow up assessment. Figures are given as number (%)

	Early drainage of cerebrospinal fluid (n=63)	*Managed conservatively (n=58)*
Hospital readmission:	44 (70)	40 (69)
1	17 (27)	20 (34)
2	13 (21)	8 (14)
≥3	14 (22)	12 (21)
Admission because of condition of central nervous system	13 (21)	11 (19)
Admission for revision of shunt	18 (29)	16 (28)
Seizures since discharge from hospital	4 (6)	11 (19)
Taking anticonvulsant drugs	5 (8)	8 (14)

As many as 54 children in the early treatment group (86%) and 49 (84%) in the group managed conservatively had abnormal neuromotor signs (relative risk 1·01; 95% CI 0·87 to 1·18); distribution and severity were similar in the two groups (table 6).

Table 7 summarises other measures of physical outcome. Although there were more babies with head circumference less than the 10th centile in the early treatment group, the mean head circumference was similar in the two groups. Visual impairment, nystagmus, and visual field deficits were less common in the early treatment group, but this difference was not significant (relative risk 0·61; 95% CI 0·35 to 1·08). Some of the children may have had retinopathy of prematurity, but this was not ascertained at the time. Overall, 67 out of 118 children tested (57%) could not see a 2 mm diameter object and 40 out of 103 (39%) of those that could see any object, had a squint.

Table 8 shows the results of the developmental paediatrician's overall assessment of out-

Table 6 Neuromotor signs at follow up. Figures are given as number (%)

	Early drainage of cerebrospinal fluid (n=63)		*Managed conservatively (n=58)*	
	All cases	*Severe cases*	*All cases*	*Severe cases*
Hypertonia affecting four limbs	14 (22)	6 (10)	14 (24)	5 (9)
Hypertonia affecting three limbs	8 (13)	1 (2)	5 (9)	1 (2)
Hypertonia affecting two limbs:				
Ipsilateral	11 (17)	3 (5)	12 (21)	1 (2)
Contralateral	8 (13)	1 (2)	2 (3)	0
Hypertonia affecting one limb	8 (13)	1 (2)	6 (10)	0
Axial imbalance	3 (5)	0	7 (12)	0
No neuromotor abnormality detected	9 (14)	0	9 (16)	0

Table 7 Physical assessment at follow up. Figures are given as number (%) unless otherwise stated

	Early drainage of cerebrospinal fluid (n=63)	*Managed conservatively (n=58)*
Mean (SE) head circumference (cm)	46·6 (3·8)	46·9 (3·6)
Head circumference centile:		
<10th centile	17 (27)	6 (10)
10–90 centile	26 (41)	33 (57)
>90th centile	20 (32)	19 (33)
Problems with sucking	3 (5)	6 (10)
Problems with choking	16 (25)	16 (28)
Excessive dribbling	6 (10)	10 (17)
Ability to see objects (at 22 cm):		
2 mm (diameter)	28 (44)	23 (40)
3–5 mm	17 (27)	10 (17)
15 mm	8 (13)	7 (12)
25 mm	3 (5)	7 (12)
No vision or sees light only	6 (10)	9 (16)
Not known	1 (2)	2 (3)
Nystagmus	7 (11)	13 (22)
Visual field deficit (but sees object)	4 (6)	7 (12)
No vision or sees light only and nystagmus or visual deficit, or both	14 (22)	21 (36)
Squint (abnormal cover test but sees object)	21 (33)	19 (33)
Distraction (hearing) test:		
Normal	36 (57)	31 (53)
Questionable	20 (32)	20 (34)
Abnormal	6 (10)	5 (9)
Head control insufficient for test	1 (2)	2 (3)

come. This, like all the neurodevelopmental assessments, was carried out without knowledge of the treament allocation. Of those children whose outcome is known (76 and 75, respectively), 58 (76%) in the early treatment group and 60 (80%) in the group managed conservatively had died or were disabled (relative risk 0·95; 95% CI 0·81 to 1·13). Although there was a suggestion of a difference between the treatment groups, with fewer children among survivors in the early treatment group having additional non-neuromotor impairments, this was not significant (relative risk 0·77; 95% CI 0·52 to 1·13). Only 18 of survivors (15%) assessed at 1 year of age were judged to be normal.

Secondary analyses, with patients stratified by whether a parenchymal lesion had been reported to be present at the time of entry to the trial, showed no difference between the trial groups in the incidence of neuromotor signs (table 9). In particular, there was no suggestion of benefit of early treatment among those who did not have parenchymal lesions at the time of entry into the trial. Nearly all the children who had parenchymal lesions at the time of entry into the trial had abnormal neuromotor signs, but there were fewer children in the early treatment group with impairments affecting other systems and this difference is marginally significant (p=0·05; relative risk 0·67; 95% CI 0·45 to 1·00).

Table 8 Final condition as judged by examiner's overall assessment, or death before assessment. Figures are given as number (%)

	Early drainage of cerebrospinal fluid (n=79)	*Managed conservatively (n=78)*
No impairment detected	9 (11)	9 (12)
Neuromotor impairment alone:	29 (37)	19 (24)
No disability	9	6
Moderate disability	4	5
Severe disability	16	8
Neuromotor plus other impairment with disability with or without DQ <70:	25 (32)	30 (38)
Neuromotor and visual	8	9
Neuromotor and sensorineural hearing loss	2	1
Neuromotor and DQ <70	4	3
Neuromotor and combination of visual impairment sensorineural hearing loss, and DQ <70	11	17
Died	13 (16)	17 (22)
No follow up assessment	3 (4)	3 (4)

DQ=developmental quotient.

Discussion

The poor outcome of children enrolled in the study has confirmed the importance of posthaemorrhagic ventricular dilatation despite its rarity. We recognised that no single unit could mount a sufficiently large trial in a reasonable length of time to evaluate early removal of cerebrospinal fluid by serial tapping. With an unprecedented degree of cooperation among neonatal units in Europe, 15 different neonatal intensive care units agreed on a protocol and enrolled infants in the trial. The planned target of 120 surviving infants was reached successfully; virtually all the infants who met the eligibility criteria were recruited. Random treatment assignment was effective in distributing the main prognostic variables between the two treatment groups (table 1). A 95% follow up at one year, with infants spread over three countries, was satisfactory, and data from the six unassessed infants who had left the country of their birth could not have altered the overall conclusions of the study.

Table 9 Outcome of survivors assessed at follow up, stratified by whether a parenchymal lesion was recorded as present at the time of entry to the trial. Results are given as number (%)

	Parenchymal lesion at time of entry		*Others*	
	Early drainage of cerebrospinal fluid (n=37)	*Managed conservatively (n=38)*	*Early drainage of cerebrospinal fluid (n=26)*	*Managed conservatively (n=20)*
Neuromotor signs				
Hypertonia affecting four limbs	11 (30)	11 (29)	3 (12)	3 (15)
Hypertonia affecting three limbs	7 (19)	5 (13)	1 (4)	0
Hypertonia affecting two limbs:				
Ipsilateral	10 (27)	11 (29)	1 (4)	1 (5)
Contralateral	2 (5)	3 (8)	8 (31)	2 (10)
Hypertonia affecting one limb	3 (8)	5 (13)	5 (19)	1 (5)
Axial imbalance	2 (5)	3 (8)	1 (4)	4 (20)
No neuromotor abnormality detected	2 (5)	0	7 (27)	9 (45)
Final overall condition				
No impairment detected	2 (5)	0	7 (27)	9 (45)
Neuromotor impairment alone:	18 (49)	12 (32)	11 (42)	7 (35)
No disability	2	4	7	2
Moderate disability	2	3	2	2
Severe disability	14	5	2	3
Neuromotor plus other impairment with disability with or without DQ <70:	17 (46)	26 (68)	8 (31)	4 (20)
Neuromotor and visual	6	7	2	2
Neuromotor and sensorineural hearing loss	0	1	2	0
Neuromotor and DQ <70	1	2	3	1
Neuromotor and combination of visual sensorineural hearing loss and DQ <70	10	16	1	1

DQ=developmental quotient.

Compliance with trial management was generally good, as shown by the differences in the number of taps and amount of cerebrospinal fluid removed during the first 14 days, and the effects of these differences on the pattern of ventricular and head enlargement (table 2). More than half the children in the early treatment group required ventricular taps to maintain the policy. Early drainage of cerebrospinal fluid had no effect on the incidence of shunt insertion subsequently, or on the number of later admissions to hospital, including those for revision of shunts (table 4). Any invasive procedure concerning cerebrospinal fluid has the potential for causing infection. Very low birthweight infants with posthaemorrhagic ventricular dilatation are particularly vulnerable, because of low concentrations of immunoglobins and complement.[20] It is well known that infants who have had shunts inserted for posthaemorrhagic ventricular dilatation have a considerable risk of infection,[13] but it is disturbing to find an appreciable number with infections of the cerebrospinal fluid after lumbar punctures and ventricular taps. Of the 157 infants enrolled, 11 (7%) developed infections of the cerebrospinal fluid before any operation had been carried out. All had had cerebrospinal fluid taps before the infection developed, and it is possible that organisms were introduced into the cerebrospinal fluid at the time of puncture. Two of these infants died with ventriculitis, and all but one of the surviving infected infants later required a permanent shunt. Although there were more infections of the cerebrospinal fluid in the early treatment group the difference was not statistically significant. This could reflect the later use of taps in the group that was managed conservatively. Non-invasive methods for reducing cerebrospinal fluid pressure, such as treatment with acetazolamide,[21] have obvious advantages in respect of the risk of infection. They are, however, of uncertain effectiveness and safety, and should be evaluated in further randomised controlled trials before widespread introduction into clinical practice.

Of the 121 children who had a follow up assessment, 103 (85%) had abnormal neuromotor signs, and in 28 (23%) hypertonia affected all four limbs (table 6). Early drainage of cerebrospinal fluid had no effect in this respect, regardless of whether or not there was a parenchymal lesion at the time of entry into the trial. There were fewer children with visual impairments and low developmental quotients in the early treatment group (table 7), and these differences were reflected in the smaller number of children who were classified as having impairments of more than just the neuromotor system (table 8). This difference was not statistically significant, however, when the total trial groups were compared.

As expected, the presence of a parenchymal lesion at trial entry was associated with a poorer long term outcome and nearly all these children had neuromotor impairments (table 9).[22] The difference in the pattern of disability between the total randomised groups seems to be limited to children with pre-existing parenchymal lesions (table 9). Children with severe motor disability, in particular, were less likely to have other types of impairment if they were in the early treatment group. Such secondary analyses should be interpreted cautiously because they are prone to wide statistical fluctuations and the observed difference is only small.[23] Nevertheless, the findings are consistent with the hypothesis that early treatment of infants with pre-existing parenchymal lesions would have little, if any, effect on motor impairment because this would reflect pre-existing periventricular damage, but would reduce other types of impairment by limiting the extent of any extension of injury to other areas of the brain.

The study provided no evidence that early treatment prevents impairments in children who do not have parenchymal lesions at the start of treatment (table 9). What is more, the overall rate of disability (46%) among these children was higher than expected.[22] In part this may be explained by the method used for diagnosis of the brain lesions. Ultrasound detects parenchymal haemorrhagic lesions accurately in the early days of life. These lesions, which are often due to secondary venous infarction, are fairly localised and are believed to lead to predominently neuromotor sequellae. By contrast, the sensitivity of detection of periventricular leucomalacia by ultrasound is low (29%),[24] and the lesions may only be recognised later. There may be multiple lesions and the type of sequellae at follow up will depend upon the site and extent of the lesions. Thus in this study periventricular leucomalacia may not have been detected until after trial entry or even missed. This explanation is supported by the identification of parenchymal lesions after trial entry in seven (15%) children and by the type of impairments found at follow up (see table 9). Nevertheless, on the basis of the findings of this study, early treatment in children who do not have parenchymal lesions at the start of treatment, does not seem to be warranted in view of the possible adverse effects of repeated drainage of the cerebrospinal fluid.

Pending the results of a further review of surviving children at the age of 2·5 years (which will include assessment of language and perception), early and repeated drainage of cerebrospinal fluid should be considered for infants with posthaemorrhagic ventricular dilatation who already have parenchymal lesions. If such management is chosen, cerebrospinal fluid taps should be performed with great care because of the risks of infection.

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On line cerebral blood flow velocity and blood pressure measurement in neonates: a new method

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Abstract

To test the hypothesis that impairment of cerebral perfusion and cerebrovascular autoregulation play a part in the pathogenesis of neurological injury in the critically sick neonate, we tested in 33 infants a small, lightweight probe and cable that are attached to the infant's skin to record cerebral blood flow velocity from the middle cerebral artery over a period of hours. This considerably reduced the amount of handling of the infant compared with conventional assessment. Captured data were analysed and displayed graphically at the cotside. The system is applicable for use on infants over a wide range of gestational ages and may give information on the complex haemodynamic changes occurring in the cerebral circulation.

Advances in neonatal intensive care, most notably in aspects of ventilatory support, have led to the prolonged survival of many extremely preterm, low birthweight infants as well as infants born at full term with various potentially life threatening disorders. A minority of surviving infants subsequently develop cerebral palsy or other neurodevelopmental problems. These may be the cause of long term financial and emotional strain on both their families and on the medical services.

The immature neonatal brain is extremely vulnerable to ischaemic and haemorrhagic injury, and the hypothesis is that disturbances of cerebral perfusion play a part in the pathogenesis of such injury.[1] This has prompted several studies that have attempted to relate circulatory indices to the subsequent development of cerebral injury to identify the major aetiological factors.[2 3] Despite these, the train of pathological events are not completely understood.

Changes in cerebral blood flow velocity correlate well with actual changes in cerebral blood flow.[4] Studies of cerebral blood flow velocity with conventional duplex scanners are limited to being done once or twice a day to minimise the handling that critically ill premature infants tolerate so badly.

This regimen has several potential drawbacks. First, there may be failure to detect both acute changes in cerebral blood flow velocity that occur between studies (for example in association with pneumothorax or haemorrhage) and more chronic changes occurring over a period of several hours. In addition, the handling that is inevitable in such studies, even if minimal, may affect the validity of the results obtained. For this reason we have assembled a system that enables us to record, analyse, and display cerebral blood flow velocity from the middle cerebral artery, as well as measurements of blood pressure, at preset or manually determined intervals with a small button transducer that is fixed to the infant's skin, thus obviating the need for repeated handling.

Methods

HARDWARE

The system is shown schematically in fig 1. The pressure front end is attached to an indwelling arterial catheter, allowing measurement of blood pressure as previously described.[5] The Doppler unit is a Doptek 4 MHz pulsed wave unit with a small button transducer (diameter 11 mm, depth 7 mm). The ultrasound intensity is below 50 mW/cm^2 spatial peak temporal average.

The use of pulsed wave ultrasound has two main advantages over continuous wave in this particular setting. First, it requires only a single, fine coaxial cable between the Doppler unit and transducer, which reduces cable drag on the transducer and makes fixation easier. Secondly, it allows the signals from the ipsilateral and contralateral middle cerebral arteries to be distinguished, even when reverse flow is present.

The remainder of the system comprises a spectrum analyser and microcomputer. The entire assembly occupies a small trolley that may be left at the cotside and does not interfere with routine nursing and medical care. The small size of the transducer is most acceptable to parents.

FIXATION

Positioning and fixing the transducer is critical because it must collect data over several hours. The Doppler unit is activated and the transducer moved over the temporal bone until the optimal signal is obtained from the ipsilateral middle cerebral atery. The transducer is then fixed in position using collodion. After fixation, the sample volume depth and length may be adjusted further to optimise the signal. Positioning and fixing the transducer normally takes three or four minutes.

ANALYSIS

Once set up the software activates the Doppler unit at preset intervals. In addition there is a manual over-ride facility, allowing the system to

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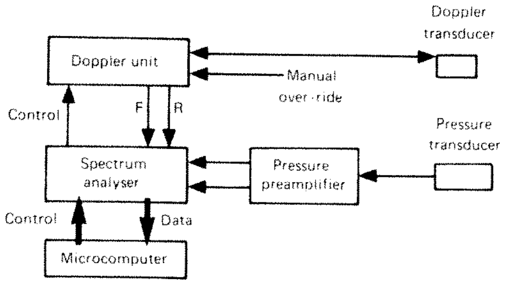

Figure 1 Schematic layout of on line system. F=forward, R=reverse.

be activated to capture changes in cerebral blood flow velocity and blood pressure associated with changes in the infant's clinical condition.

After activation, 12 seconds of blood pressure and spectrum analysed Doppler data are acquired by the microcomputer. The Doppler unit is then switched off. A maximum frequency envelope is derived from the Doppler data,[6] and after the data are split into individual cardiac cycles, various measurements—including mean velocity (and its coefficient of variation), received Doppler power, and resistance area produced (RAP=mean blood pressure/mean blood flow velocity), an index of cerebrovascular resistance—are calculated.[7] The results are written to disk and displayed graphically at the cotside.

Results

We have used the system on 33 infants. Details of the infants studied are given in the table. Informed consent was obtained before each study, and parents were encouraged to be present for all or part of the study.

The gestational age (24–41 weeks) and weight range (750–3510 g) of the group studied is representative of the population seen in most neonatal intensive care units. The average length of study was 4·5 hours (range 2–8), and in most cases (29 of 33 studies) it was decided electively to discontinue the study. In four cases the infant's own movements or the nurses turning the baby caused appreciable degradation in signal quality to the extent that further recordings were not possible without reapplication of the transducer. The longest period of continuous monitoring without reapplying the transducer was 48 hours.

Studies were planned to begin immediately after nursing care had been given to minimise periods of handling. The study was discontinued if a change in position at the next care resulted in the infant's head lying on the Doppler transducer, which we feared might damage the sensitive skin. Alternatively the transducer could be reapplied to insonate the contralateral middle cerebral artery.

Details of infants studied

Gestation (weeks)	*No of infants*	*Weight range (g)*
<30	15	750–1470
30–34	11	1230–1710
>34	7	1920–3510

The main problem encountered so far has been to maintain the transducer alignment and hence the quality of the Doppler signal for prolonged periods. The system works best in relatively inactive or therapeutically paralysed infants who are receiving minimal nursing and medical handling. This group of infants tends to be the most critically ill and therefore most at risk of developing neurological complications. It is envisaged that in practice the transducer should be checked every four hours and reapplied if necesseary in much the same way that transcutaneous gas monitors are used. In this way monitoring can be continued for days.

We have used the system to study normal variations in cerebral blood flow velocity and blood pressure and have also recorded the changes that occur in response to drugs and to ventilatory adjustment.

A typical trace from an infant of 29 weeks' gestation being ventilated for respiratory distress syndrome is shown in fig 2. Several features on the traces are worthy of note. Inadequate flushing of the indwelling arterial line resulted in damping of the blood pressure trace (*point a, upper trace*). Any activity on the part of the infant or any handling resulted in a change in cerebral blood flow velocity, in this case an increase (*point b, middle trace*). This highlights the inadequacies of intermittent sampling of cerebral blood flow velocity with conventional equipment.

Carbon dioxide is a potent vasodilator, and the cerebral vasculature is extremely sensitive to fluctuations in arterial carbon dioxide tension ($PaCO_2$), that are mediated by changes in the

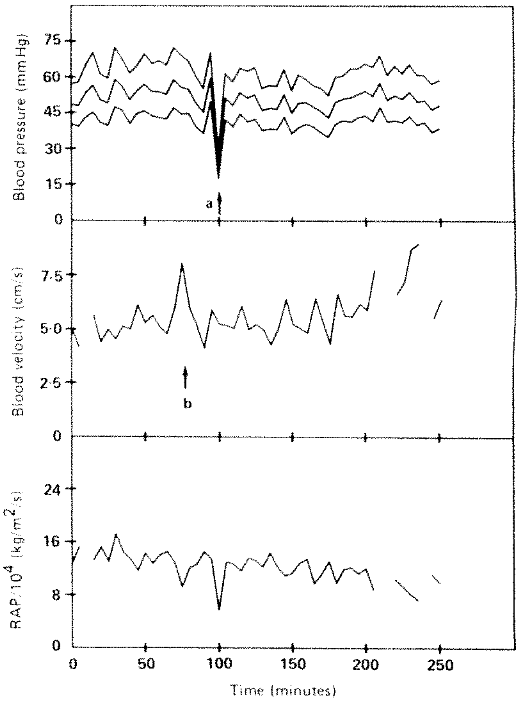

Figure 2 Normal fluctuations in cerebral blood flow velocity. RAP=resistance area produced (mean blood pressure/mean blood flow velocity).

pH of the cerebral interstitial fluid. A reduction in the inspiratory pressure of an infant being ventilated for respiratory distress syndrome resulted in an immediate rise in cerebral blood flow velocity (fig 3, *point c*). Repeat arterial blood gas sampling (*point d*) confirmed the rapid rise in $PaCO_2$, and returning the ventilator pressure to its original setting restored the carbon dioxide tension and cerebral blood flow velocity to their previous levels. A similar effect may be obtained by inserting a dead space into the ventilator circuit (*point e*), which causes a rise in $PaCO_2$, and then removing it (*point f*).

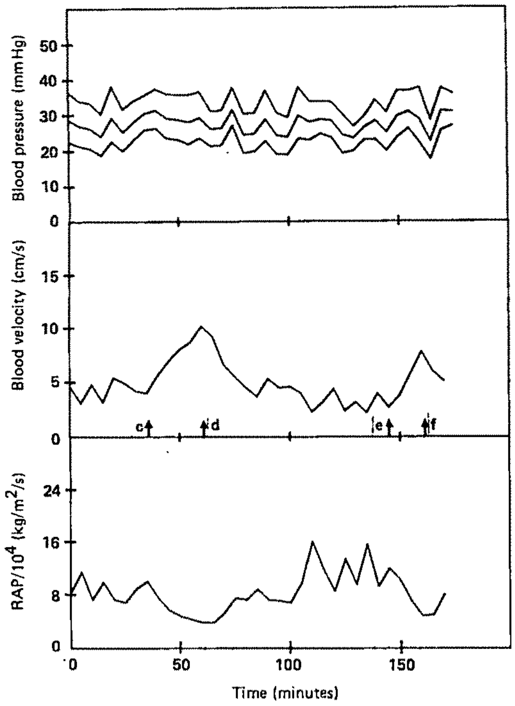

Figure 3 Effect of carbon dioxide on cerebral blood flow velocity. RAP=resistance area produced (mean blood pressure/mean blood flow velocity).

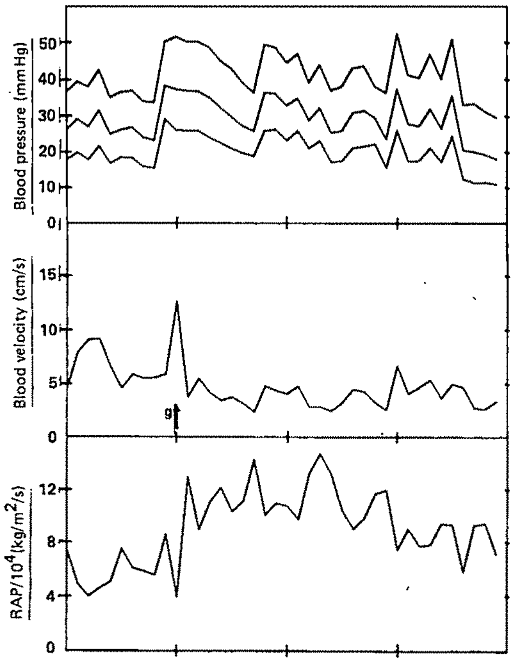

Figure 4 Effect of indomethacin on cerebral blood flow velocity. RAP=resistance area produced (mean blood pressure/mean blood flow velocity).

Several drugs that may have profound effects on the circulation are used in neonatal intensive care. For example, indomethacin is used in the medical management of patent ductus arteriosus. Giving indomethacin (fig 4, *point g*) resulted in a sustained fall in cerebral blood flow velocity and a sustained rise in resistance area product, which presumably indicates vasoconstriction.[8] The rise in cerebral blood flow velocity immediately before the drug was given was caused by handling the infant.

Discussion

We have observed no local adverse effects from fixation of the transducer and believe it to be safe in terms of power output.[9] The system is simple, can be rapidly set up, and may be used to collect data in two ways. First, it allows the study of both normal and pathological variations in cerebral blood flow velocity in critically ill neonates over periods of several hours and relates these to changes in blood pressure, while adhering to the principle of minimal handling. More continuous measurement of changes in cerebral blood flow velocity is more likely to detect important changes that correlate with the development of ischaemic or haemorrhagic cerebral events.

Secondly, it may be used to monitor rapid changes in cerebral haemodynamics resulting from ventilatory or pharmacological manipulation, or from clinical deterioration. During the course of evaluating this equipment it has become apparent that wide fluctuations in cerebral blood flow velocity may occur as a result of handling the infants. In non-paralysed infants this may be caused, in part, by breathing asynchronously with the ventilator. Perlman *et al* have suggested that fluctuating cerebral blood flow velocity causes intraventricular haemorrhage,[10] but we suspect that merely placing the ultrasound probe on the infant's head is enough to cause these abnormal patterns. It is not yet clear whether fluctuations in cerebral blood flow velocity as described by Perlman *et al*, if present in infants who are not disturbed, are as important as those induced by handling. Our equipment obviates the need for repeated handling, allows more detailed study of the ill infant's spontaneous interaction with the ventilator, and will help us to determine the answer to this important question.

This system will also allow the investigator to assess the effect of spontaneous changes in blood pressure on cerebral blood flow velocity in an attempt to provide further evidence for the failure of cerebrovascular autoregulation in the pathogenesis of neurological injury in the critically ill newborn infant. It may help us to formulate and monitor management regimens aimed at reducing the incidence of such injury.

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Reproducibility of measurements of cardiac output in newborn infants by Doppler ultrasound

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Abstract

Interobserver reproducibility in deriving cardiac output by measuring aortic blood flow velocity and diameter with imaging and Doppler ultrasound was investigated in 20 healthy infants born at full term. Aortic diameter was measured in three ways. Mean blood flow velocity was measured at three sites with both continuous wave and pulsed Doppler. Two observers carried out each study independently. Intraobserver reproducibility was investigated in 12 infants using the suprasternal site for measuring blood flow velocity. The most reproducible determination of cardiac output was found when the suprasternal site with continuous wave Doppler was used for measurement of blood flow velocity and M mode trailing edge to leading edge echocardiography was used for diameter. Normal mean (2 SD) cardiac output is 231 (77) ml/kg/min.

Technical difficulties in measuring aortic diameter accurately limit direct comparison between infants.

Reliable measurement of cardiac output in newborn infants would be useful in various ways. Non-invasive methods such as impedance cardiography,[1 2] or selected M mode echocardiographic indices,[3] have been available for over 10 years but have not become widely accepted. Doppler ultrasound has more recently been introduced. Theoretically this should be ideal, being both non-invasive and repeatable.

Measurement of cardiac output with Doppler ultrasound requires measurement of aortic cross sectional area and blood flow velocity. Alverson *et al*,[4–7] Mellander *et al*,[8] and Walther *et al*[9] have pointed out the limitations in measurement of diameter and there is no universally accepted method. Walther used M mode leading edge to leading edge echocardiography (fig 1),[9] and Alverson *et al* used M mode trailing edge to leading edge echocardiography.[4–7] Mellander *et al*, who used both the cross sectional image for internal aortic diameter and M mode trailing edge to leading edge method, found the M mode more satisfactory.[8] As the area is derived from the square of the radius, any error in the measurement of the diameter will be magnified.

Accurate assessment of blood flow velocity requires the Doppler beam to be aligned with the long axis of the aorta, and any deviation will result in an underestimation that is related to the cosine of the angle. In practice, providing the angle is less than 15°, the error will be less than 3% (fig 2). The suprasternal site has generally been used but this may not be ideal, especially in infants who are being ventilated and in whom this site may be inaccessible; either the apical window or the subcostal site may be preferable and geometrically better aligned.

Velocity can be measured by two different techniques; pulsed Doppler obtains information about flow at a predetermined depth and has usually been used, and the alternative, continuous wave, has no depth resolution but may be easier to use and be more reproducible.

Zero crossing detectors[4–7 9] or mean velocity estimators have been used in previous studies, but these have definite limitations.[10] Thus although discrete Fourier transformation is used in all modern Doppler systems, the results of measuring cardiac output in the newborn have not been fully investigated. In addition the advent of coloured Doppler enables a more accurate diagnosis of an arterial duct, allowing such patients to be excluded from the study.

We know of no other study in newborn infants that directly compares reproducibility in measurement of cardiac output at the three standard sites using both pulsed and continuous wave Doppler, and using the three different quoted methods for measuring aortic diameter. We therefore report the results of a study using Doppler spectral analysis.

Methods

The first part of the study determined interobserver differences in the measurement of cardiac output. We studied 20 healthy infants, whose gestational age range was 37 to 41 weeks, who were between 2 and 7 days old at the time of the study, and whose weights ranged from 2380 to 4020 g. They were all quiet at the time of the study.

Measurements were made using a Vingmed CFM 700 Duplex ultrasound scanner with a 5 MHz probe, which uses discrete Fourier transformation. Spectral signals were recorded at the time of study and the audio signals were later analysed by a Doptek spectrum analyser, which can edit and calculate mean blood flow velocity and analyse a sequence of cardiac cycles. For this study at least 10 cycles were analysed for each measurement of mean blood flow velocity. A ductus arteriosus was excluded using colour Doppler.

Observations were made either by a physiological measurement technician who was experienced in imaging and Doppler ultrasound, or by

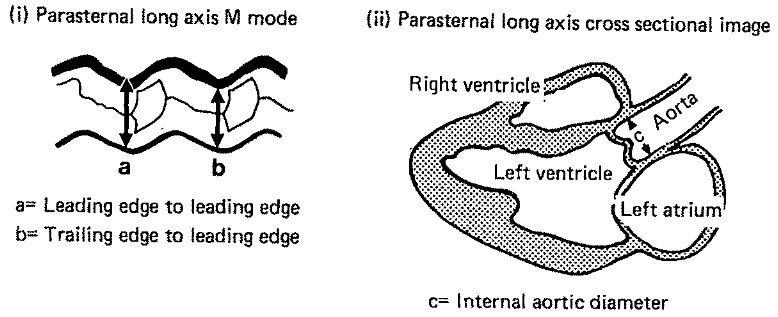
(i) Parasternal long axis M mode
a
b
a= Leading edge to leading edge
b= Trailing edge to leading edge
(ii) Parasternal long axis cross sectional image
Right ventricle
Aorta
c
Left ventricle
Left atrium
c= Internal aortic diameter

Figure 1 Methods of measuring aortic diameter.

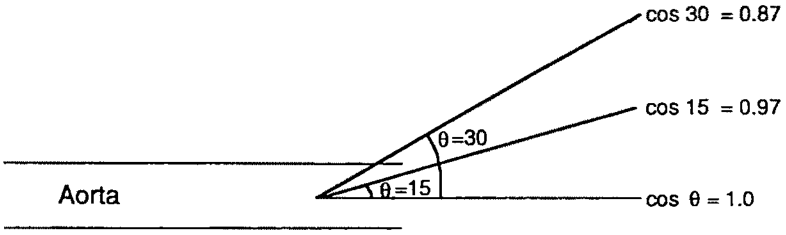

Figure 2 Effect of alignment of beam on underestimation of blood flow velocity. Velocity is calculated from Doppler equation $ft-fr=2\,ft\,V\cos\theta/c$*: where ft is transmitted frequency, fr is received frequency, c is ultrasound propagation velocity, V is blood flow velocity, and* θ *is angle between ultrasound beam and direction of flow. Angle* θ *cannot be measured accurately and is assumed to be zero. Increasing angle* θ *causes progressive underestimation of velocity.*

a neonatologist trained in the technique by cardiologists. The technique was acquired over several months.

Aortic diameter was determined at end diastole from a parasternal long axis view in three ways: firstly, using the M mode leading edge to leading edge technique; secondly, from the M mode trailing edge to leading edge method; and, thirdly, direct from the cross sectional image measuring the internal aortic diameter (fig 1). Measurement was taken as the average of three cycles from a hard copy. The measurement of the diameter was then repeated independently by a second observer.

Aortic Doppler signals were recorded for later analysis from three sites: suprasternal, apical, and subcostal, using both continuous wave and pulsed Doppler. The operator used imaging ultrasound to align the beam roughly in the correct anatomical site, then adjusted the beam and the depth to give the best possible signals independent of the image. Measurements were then repeated independently by a second observer. For each infant cardiac output was calculated from these signals at each of the three sites using both types of Doppler.

The second part of the study assessed intraobserver differences in the measurement of cardiac output. Twelve infants weighing between 1000 and 3900 g had both pulsed and continuous wave Doppler measurements of mean blood flow velocity made at the suprasternal site. Diameter was measured using the M mode trailing edge to leading edge method. Two independent measurements of cardiac output were made by the same observer 30 minutes apart.

STATISTICAL METHODS

Each observation of a particular patient should be thought of as arising in the following manner: an overall population mean, plus a patient component (relative to the mean), plus a between observer 'measurement error', plus a within observer 'measurement error' component. A measurement where the last two components are small compared to the first component (that is, the 'between patient' variability) is a reproducible measurement.

In the analysis of the data from two observers the first step in the investigation is to find out whether there is a significant degree of bias between observers and, if there is, the measurement is considered unsuitable for general use. If there is little or no bias between observers, all the above components of variability are estimated and the percentage of the total variability (that is, the 'sum' of all three components) that is constituted by the between/within observer components is reported.

Also reported are the standard deviations of a single measurement based on the 'sum' of all three components—that is, an estimate of the variability likely to be seen in an observation from a randomly chosen patient measured once by a single observer.

In the analysis of replicate measurements by the same observer one can estimate the 'within observer' variability only, and then report what percentage of the total variability this is.

All comparisons of sites, methods, or interobserver biases are made on repeated measures of analyses of variance and, where applicable, paired *t* tests.

Results

DETERMINATION OF INTEROBSERVER REPRODUCIBILITY

Aortic diameter

None of the three measurements of aortic diameter showed any significant interobserver bias (table 1). Clearly the best measurement was M mode trailing edge to leading edge echocardiography, which showed roughly half the between/within observer variability of the other two measurements—that is, only 25% of the total variability in a single measurement of aortic diameter taken from the M mode trailing edge to leading edge can be attributed to between observer or within observer error (table 1).

An overall summary of the means and estimated standard deviations from a single obser-

Table 1 Interobserver variability in measuring aortic diameter

Measurement	*Mean (SEM) interobserver difference (mm)*	*Estimated SD caused by observers (% of total variance) (mm)*
Cross sectional inner aortic diameter	0·04 (0·15)	0·47 (51)
M mode trailing edge to leading edge	0·00 (0·11)	0·35 (25)
M mode leading edge to leading edge	0·07 (0·16)	0·51 (46)

None of these measurements shows significant interobserver bias.

vation for the three measurements is shown in table 2. M mode trailing edge to leading edge echocardiography is the most reproducible measurement and henceforth used to calculate cardiac output.

Cardiac output

Table 3 shows both Doppler measurements for the subcostal site and the pulsed Doppler for the apical site in which there are significant interobserver biases making them unsuitable for general use until some explanation of, and correction for, these biases is forthcoming. There is little to choose among the other three site/type combinations. Because of the problem of interobserver bias at the apical site for pulsed Doppler values, however, it seemed more sensible to use the suprasternal site as a more reliable site for measurements of cardiac output. The second part of the study, therefore, investigated within observer variability at this site.

An overall summary of all site/type combinations and the resulting repeated measures analysis of variance showed no significant site difference ($p<0·05$) but among sites (table 4), there was a significant difference between Doppler types in that continuous wave measurements were between 5 and 25 ml/kg/min lower than the corresponding pulsed measurements.

Table 2 Summary of measurements of aortic diameter in 20 infants

Measurement	*Estimated population mean (mm)*	*Estimated SD based on a single observation (mm)*
Cross sectional inner aortic diameter	7·77	0·65
M mode trailing edge to leading edge	8·14	0·70
M mode leading edge to leading edge	9·94	0·74

Table 3 Interobserver variability in measuring cardiac output given as ml/kg/min

Site/ Doppler type	*Mean (SEM) interobserver difference*	*Estimated SD caused by observers (% of total variance)*
Subcostal:		
Pulsed	28·5* (8·9)	Interobserver bias
Continuous	22·9* (9·7)	Interobserver bias
Apical:		
Pulsed	32·6* (13·4)	Interobserver bias
Continuous	13·9 (9·9)	32·8 (55·2)
Suprasternal:		
Pulsed	3·9 (11·3)	35·8 (56·43)
Continuous	10·6 (8·9)	29·0 (57·2)

*Significant interobserver bias (p=0·05).

Table 4 Summary of measurements of cardiac output in 20 infants (ml/kg/min)

Site/ Doppler type	*Estimated population mean*	*Estimated SD based on a single observation*
Subcostal:		
Pulsed	244·0	Interobserver bias
Continuous	230·8	Interobserver bias
Apical:		
Pulsed	252·9	Interobserver bias
Continuous	227·7	44·2
Suprasternal:		
Pulsed	241·4	47·6
Continuous	230·9	38·3

DETERMINATION OF INTRAOBSERVER VARIABILITY

Continuous wave Doppler measurements for this observer were more reproducible than pulsed Doppler (table 5); the within observer variability constituted only 18% of the total variability in a single measurement, which is considerably less than the 57% contribution from the combination of between observer and within observer variability reported in the first part of the study.

The overall summary of the two measurements for this part of the study is shown in table 6, and the sample of 12 infants seems to come from a population with mean cardiac outputs about 20 ml/kg/min lower than that of the 20 infants in the first part of the study. This may be attributable to lower gestational ages in this sample of 12. Again, however, there is a consistent difference of about 10 ml/kg/min between cardiac output measured by the two Doppler types.

In summary, therefore, when taking into account both experiments it seems reasonable to conclude that the continuous wave Doppler measurements taken at the suprasternal site are the most reproducible of all those considered, in that they show no significant interobserver bias, and are repeatable by a single observer.

MINIMAL DETECTABLE CHANGE IN CARDIAC OUTPUT

These results indicate that using continuous wave Doppler at the suprasternal site, and assuming the same diameter for each measure-

Table 5 Intraobserver variability in measuring cardiac output at suprasternal site (mg/kg/min)

Doppler type	*Estimated SD caused by intraobserver variability (% of total variance)*
Pulsed	19·1 (29·0)
Continuous	16·5 (18·4)

Table 6 Summary of measurements cardiac output at suprasternal site in 12 infants for one observer (mg/kg/min)

Doppler type	*Estimated population mean*	*Estimated SD based on a single observation*
Pulsed	220·9	35·5
Continuous	209·3	38·5

Table 7 Calculated effect of error in measurement of aortic diameter

Actual diameter (mm)	*Measured diameter (mm)*	*Error in cardiac output (%)*
11	10	17
9	10	23
9	8	21
6	7	35
5	6	43

ment, the minimum difference in cardiac output that could be regarded as significant would be 90 ml/kg/min if made by two independent observers, or 45 ml/kg/min if made by the same observer on two occasions.

Discussion

Although pulsed Doppler has been used principally to measure cardiac output, Hatle used continuous wave Doppler successfully in neonates and young children for assessing aortic blood flow velocities.[11] Nishmura *et al* used continuous wave Doppler in adults to measure cardiac output, and found it correlated well with invasive methods.[12] Our results indicate that continuous wave is more reproducible than pulsed wave Doppler in the measurement of cardiac output by two independent observers. This may be because of the different depths being chosen by the two observers in attempting to obtain the best signals each time. We also found that roughly half the observed variance within the population of infants studied when measured by independent observers was the result of 'observer error'.

In comparing values obtained by the same observer the variability in both types of Doppler is less pronounced, and may reflect the same depth being chosen each time. We confirm the finding of Isken *et al* (VHA Isken, A Leonhardt, O Lindenkamp. Pulsed Doppler determination of cardiac output during the early neonatal period: validation of method using apical window. Presented to Internationales Symposium in Munster, June 1988, Neve Aspekte des Blurkreis laufes urd Sau erstofftransportes bei Fluhgeborenen) who obtained similar values when comparing the apical window and suprasternal sites. Our own results show similar values from the three sites, but greater variability in the subcostal and apical sites compared with the suprasternal site, as well as interobserver biases.

We found that continuous wave Doppler gave significantly lower values than pulsed Doppler at each site; this was presumably because more lower velocity signals are recorded by the continuous wave beam. In some infants it was technically difficult to obtain clear continuous wave signals because of interference from other high velocity signals, and in these infants pulsed Doppler should be used. For pulsed Doppler studies, the equipment must be a duplex system, with both imaging and Doppler combined; this is available in almost all modern ultrasound equipment. Free standing Doppler systems are available (Doptek, Vingmed), and although these are difficult to use with pulsed Doppler they should give satisfactory results with continuous wave Doppler. Colour Doppler has no advantage in aligning the beam and is not neccessary for measuring cardiac output.

Using the suprasternal site and continuous wave Doppler, and measuring the diameter using M mode trailing edge to leading edge echocardiography, our results confirm those of Alverson *et al*,[4–7] Mellander *et al*,[8] and Walther *et al*.[9] We are surprised that the results obtained by Walther *et al* are similar to ours, as well as to those of Mellander *et al* and Alverson *et al*, because they calculated aortic diameter using the leading edge to leading edge method; Mellander *et al* and Alverson *et al* used the trailing edge method, and we found a 2 mm difference in the leading edge compared with the trailing edge method for measuring diameter. The effect of errors in measuring diameter can be calculated (table 7).

Alverson *et al* and Walther *et al* used zero crossing detectors, which are now outdated; we used discrete Fourier transformation. This is much less gain dependent, more widely available, and used in all modern Doppler machines (which also contain the software for calculating cardiac output). This study also has the advantage of colour Doppler, which enabled more accurate exclusion of patent ductus arteriosus and thus eliminated any possible error related to the resulting increased aortic flow.

Our study was designed to determine the most reproducible method of measuring cardiac output. To determine the method that gives the most accurate result, direct comparison with invasive techniques would be neccessary. Unfortunately there seems to be no 'gold standard' for direct comparison. Powner and Snyder[13], in reviewing six thermodilution cardiac output systems, pointed out that these may be liable to errors of up to 20%. Despite this Alverson[7] and Mellander *et al*[8] both compared invasive methods for determination of cardiac output using the Fick principle with Doppler ultrasound in infants and older children, and found good correlation; our results with Doppler are similar.

The main limitation of the technique seems to be in accurate measurement of aortic diameter, and as can be seen from our calculations (table 7) a small error may produce a relatively large error in the calculation of cardiac output, especially in preterm infants with small aortic diameters. Although direct comparison among different infants may give rise to considerable error, serial measurements within the same infant made by the same observer will reduce this error and this must be the main use of the technique. The result may, however, be an underestimation because the technique does not measure coronary blood flow and this may account for between 5% and 10% of the total cardiac output. It also assumes that aortic diameter does not change throughout systole.

Others have found Doppler ultrasound useful in monitoring serial changes in infants with myocardial dysfunction,[14] various arrhythmias, and in those receiving treatment for ductus arteriosus.[7]

We conclude that Doppler ultrasound is a useful technique for serially measuring cardiac output in an individual infant. The most reproducible results are obtained using diameters measured by M mode trailing edge to leading edge echocardiography, mean blood and flow velocities measured by continuous wave Doppler at the suprasternal site; the measurements should be made by the same observer on each occasion. Results obtained using spectral analysis are similar to those obtained using zero crossing detectors.

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Prenatal measurement of cardiothoracic ratio in evaluation of heart disease

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Abstract

The cardiothoracic ratio was measured in 410 normal fetuses and in a group of 73 fetuses with functional or structural heart disease. In normal fetuses it was fairly constant throughout pregnancy, but of those with congenital heart disease it was raised in cases of Ebstein's anomaly, tricuspid dysplasia, atrioventricular septal defect, and complete heart block. In some forms of congenital heart disease, however, it was within the normal range. There was a significant positive correlation between the cardiothoracic ratio and fetal hydrops in the group of 15 fetuses with supraventricular tachycardias. In these fetuses the cardiac size decreased significantly once the fetus reverted to sinus rhythm after the mother had been treated. Measurement of the cardiothoracic ratio is essential in the evaluation of fetal hydrops, as an increased value may point to the diagnosis of an intermittent fetal tachycardia if the fetus is assessed during a period of sinus rhythm.

The measurement of this index forms a part of the complete prenatal evaluation of structural heart disease. The degree of cardiomegaly may provide useful information about secondary lung compression or cardiac failure and therefore assist in giving an accurate prognosis for postnatal survival.

The ratio of the size of the heart to that of the thorax on a plain chest radiograph has long been used as an index of cardiac disease or heart failure in both paediatric and adult cardiology. Many forms of congenital heart disease, however, do not show cardiac enlargement but have abnormal measurements of individual chambers. For that reason cardiologists evaluating the fetal heart have measured the ventricular or atrial size by echocardiography throughout gestation to provide a normal range of measurements for comparison with those with suspected malformations.[1,2] The cardiothoracic ratio has been assessed in normal patients[3] and used to evaluate pulmonary volume prenatally in thoracic hypoplasia, but this measurement has not to our knowledge been used to assess the heart in the presence of structural malformation.[4] For this reason, we measured the cardiothoracic ratio throughout pregnancy in normal fetuses and in groups of fetuses with specific functional or structural heart disease.

Subjects and methods

Since the beginning of 1985 we have examined more than 2000 pregnancies. All the echocardiograms have been recorded on videotape. The records of 410 examinations, selected retrospectively, have been used to acquire the normal data for the cardiothoracic ratio. The selection criteria for these pregnancies were: that the gestational age had been assessed by early ultrasound examination, the pregnancy was uneventful, the neonatal outcome was normal, there was a suitable thoracic view for measurement on the videotape record, and there was no sign of congenital heart disease.

A Hewlett-Packard 77020A sector scanner with 5·0 and 3·5 mHz probes was used. The thoracic view was considered suitable for measurement when the whole thorax was seen on the screen, a good four chamber view was achieved, and there was a complete rib and no abdominal contents in the frame. All the mesurements were performed in diastole (fig 1). The circumference of the heart and the circumference of the thorax were measured and ex-

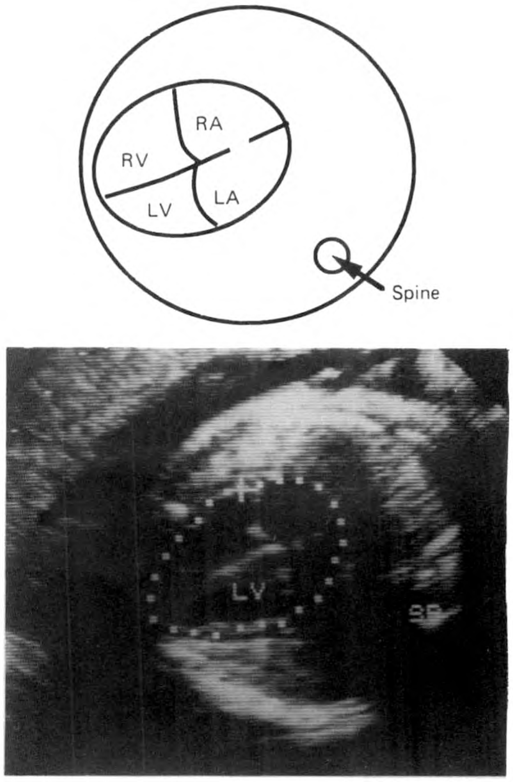

Figure 1 A thoracic section suitable for measurement. The cardiac circumference is indicated. RA=right atrium, LA=left atrium, LV=left ventricle, and RV=right ventricle.

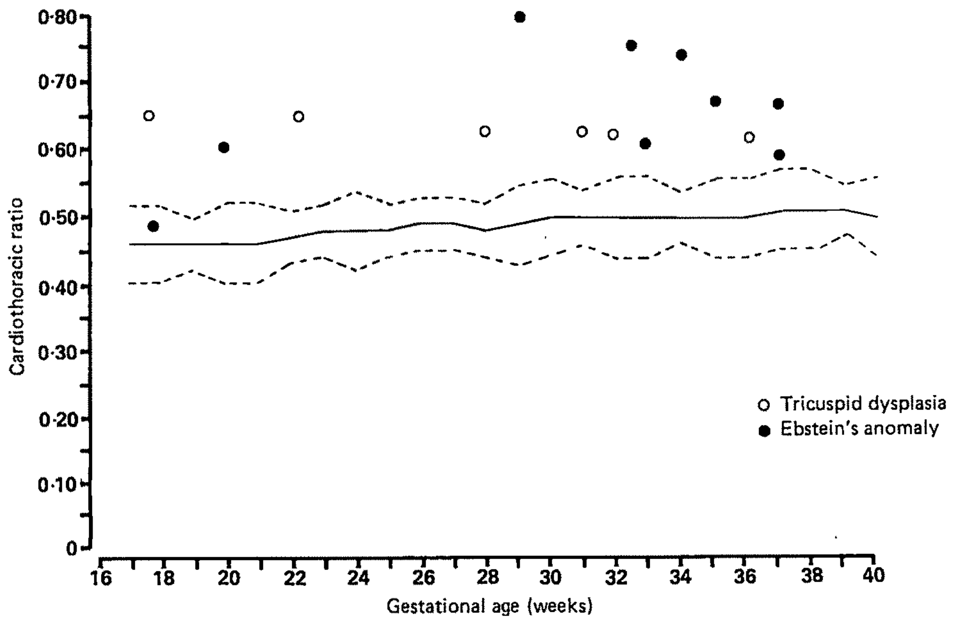

Figure 2 The cardiothoracic ratio in 15 cases of tricuspid valve abnormality compared with the normal range.

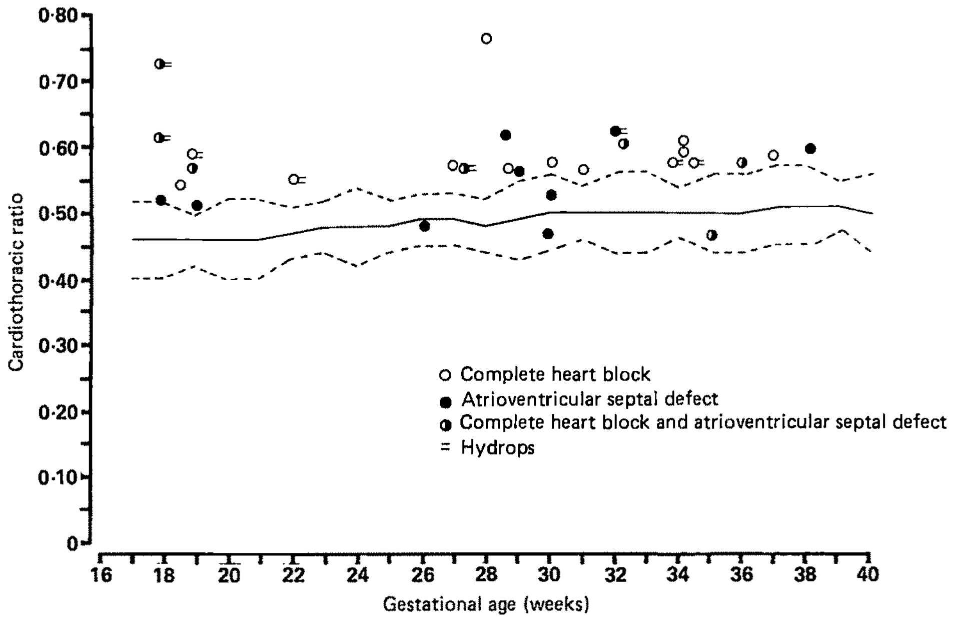

Figure 3 With only one exception, all cases of complete heart block showed cardiomegaly on the cardiothoracic ratio whether or not there was associated fetal hydrops.

pressed as a ratio. The mean was taken of two different measurements in two different frames.

Four groups of fetuses were studied including 15 with Ebstein's anomaly or tricuspid dysplasia, 29 with complete heart block, or an atrioventricular septal defect, or both, 15 with atrial tachycardias, and a further 14 with various forms of congenital heart disease.

Statistical analysis was by Student's nonpaired two tailed t test.

Results

The cardiothoracic ratio was fairly constant throughout pregnancy, with a slight increase from 0·45 at 17 weeks to 0·50 at term. The normal range with 95% confidence interval is indicated in the graphs of the abnormal groups (figs 2 to 5).

In the cases with tricuspid valve abnormality, there was a high cardiothoracic ratio in 14 of 15 cases (fig 2). One case with a mild form of Ebstein's anomaly seen in early pregnancy was within the normal range.

Within the second group there were 13 cases of isolated complete heart block, nine cases of atrioventricular septal defect, and seven with both an atrioventricular septal defect and complete heart block (fig 3). The cardiothoracic ratio was increased in 24 fetuses. In all the cases

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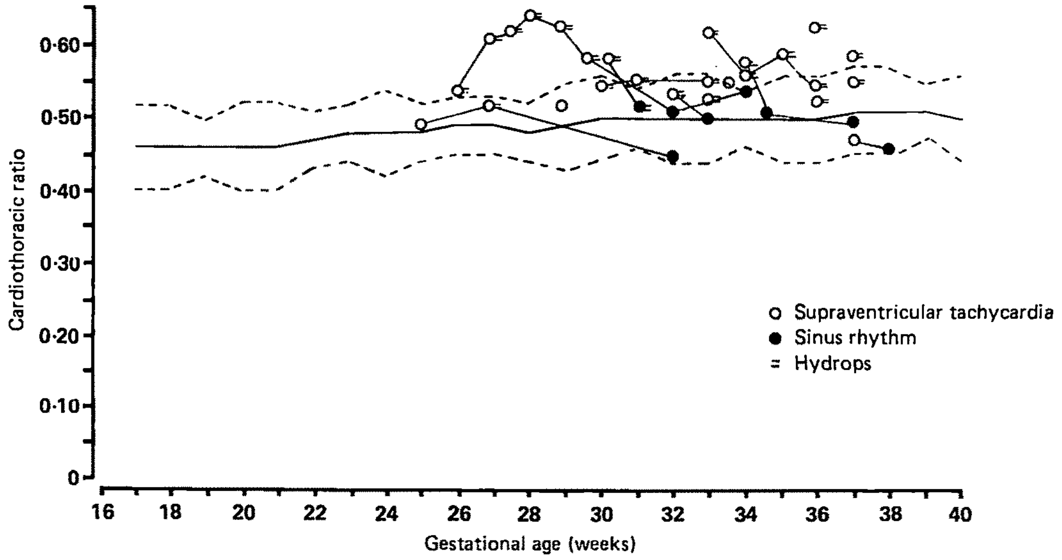

Figure 4 Serial measurement of the cardiothoracic ratio in cases of fetal tachycardia showing correlation between the presence of hydrops and cardiac enlargement. Some fetuses remained hydropic after conversion to sinus rhythm and after the cardiothoracic ratio became normal.

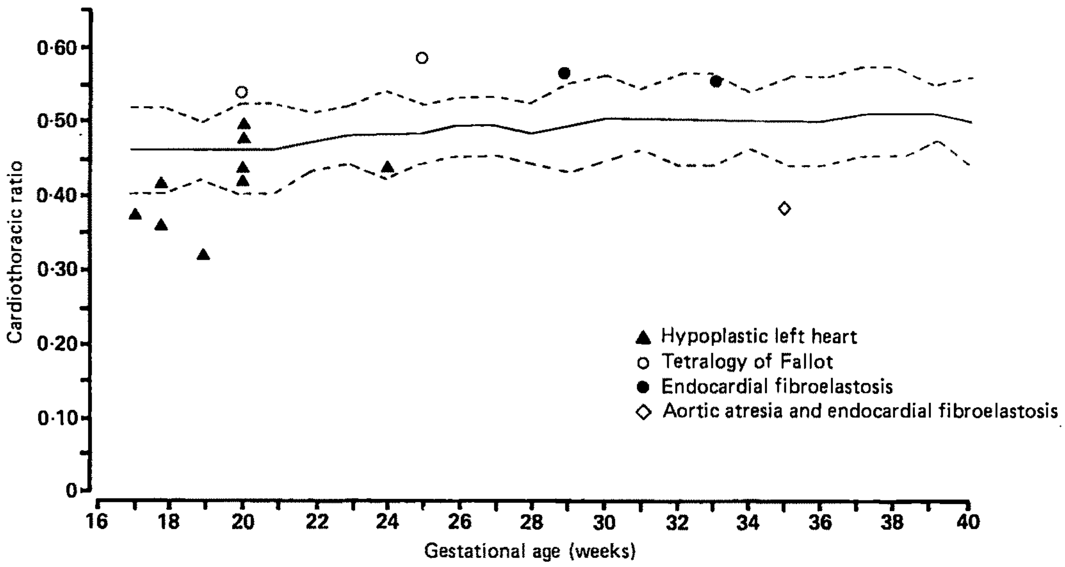

Figure 5 An assorted group of cases of fetal heart disease compared with the normal range. No consistent pattern of cardiothoracic ratio measurement was found.

complicated by fetal hydrops the ratio was increased, but there was no clear difference in cardiac size between the hydropic and non-hydropic cases with complete heart block.

In those cases with a fetal tachycardia, 34 measurements were made (fig 4). Not all those complicated by fetal hydrops were above the normal range, but the mean (SD) cardiothoracic ratio for the hydropic fetuses was 0·55 (0·34) against 0·49 (0·3) for the non-hydropic cases ($p<0·01$). Nine measurements were made after the fetuses had reverted to sinus rhythm because the mother was receiving treatment. In these cases, the ratios fell significantly ($p<0·01$) from the estimates before treatment (0·50 (0·28) compared with 0·55 (0·37)).

In the last group cases with different forms of congenital heart disease were evaluated (fig 5).

No clear pattern of cardiac size is characteristic of all forms of congenital heart disease.

Discussion

A suitable view for estimation of the cardiothoracic ratio can readily be obtained in all patients. Some forms of heart disease are particularly associated with cardiac enlargement—for example, Ebstein's anomaly and tricuspid dysplasia. This form of heart disease is commonly associated with early neonatal death from lung hypoplasia. The underdevelopment of the lungs occurs as a result of lung compression, which is secondary to often gross cardiomegaly. It is possible that the degree of cardiac enlargement in these cases may correlate with the postnatal outcome. Expansion of these data may answer this.

There are some forms of heart disease where the heart is not enlarged prenatally, or is below the normal range of cardiac size—for example, hypoplastic left heart syndrome. In all cases but one in complete heart block the heart was enlarged, whether or not there was associated fetal hydrops.

Where cardiac failure develops as a result of an intrauterine tachycardia, obvious cardiac enlargement is detected by measurement of the cardiothoracic ratio. A fetus can remain hydropic, however, after a supraventricular tachycardia has been controlled and the heart size has returned to normal. This may indicate that the fetus has difficulty in clearing peripheral fluid collections even after cardiac function has returned to normal.

In summary, measurement of the cardiothoracic ratio is simple to do and should be part of the complete prenatal evaluation of structural or functional heart disease. The degree of increase of this index from the normal range may assist in predicting the extent of lung compression and the severity of heart failure.

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Postnatal circulatory adaptation in healthy term and preterm neonates

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Abstract

Thirty seven healthy infants (18 born at full term and 19 preterm) were studied serially with cross sectional and Doppler echocardiography to compare their postnatal circulatory adaptation. Pulmonary artery pressure was assessed by its inverse relationship with the ratio of pulmonary artery time to peak velocity and right ventricular ejection time measured from Doppler waveform. Patency of the ductus arteriosus and interatrial shunting were assessed by imaging and Doppler ultrasound. The ratio rose after birth in all infants; it rose more slowly in the preterm infants. After 6 hours of age the mean was significantly less in the preterm group, the greatest difference being between 25 and 36 hours. By 73 to 96 hours the difference was no longer significant. There was a trend towards later ductal closure in the preterm infants but this was not significant. Atrial shunting level varied, but some left to right shunting was seen in all infants satisfactorily studied. Pulmonary artery pressure seems to fall more slowly after preterm birth even in the absence of respiratory problems, but ductal shunting persisting for more than three days is unusual in healthy preterm infants.

Two dimensional and Doppler echocardiography provide a non-invasive method for studying the circulatory adaptation to extrauterine life in humans. The ductus arteriosus and foramen ovale can be seen, and analysis of Doppler wave forms has lead to the development of indirect methods for assessing pulmonary artery pressure. The ratio of pulmonary artery time to peak velocity (TPV) and right ventricular ejection time (RVET) as measured from the Doppler waveform has a close inverse correlation with mean pulmonary artery pressure. This correlation has been validated against direct catheter pressure measurement in children and adults,[1–3] and is a good way of studying pulmonary haemodynamics in the newborn. Ultrasound studies of the transitional circulation in infants born at full term have shown that the TPV:RVET ratio rises significantly over the first 24 to 36 hours of life with ductal closure invariably complete by 48 hours.[4–6] Postnatal catheter studies using direct pressure measurements have shown a similar rate of fall both in pulmonary artery pressure and in time taken for complete ductal closure.[7] No such measurements have been published for healthy preterm infants. Such data is important for comparative purposes, as one of the potential uses of the TPV:RVET ratio is to study pulmonary artery pressure in infants with respiratory problems. In this study, using two dimensional and Doppler echocardiography, the rate of circulatory adaptation to extrauterine life was compared between healthy infants born at full term and those born prematurely.

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Patients and methods

All subjects were studied with an ATL Ultramark 4 scanner and range gated pulsed Doppler. Cross sectional imaging was done with a 7·5 MHz transducer incorporating a 5 MHz pulsed Doppler crystal.

The procedure for each study was as follows: the pulmonary artery was visualised from the parasternal long axis view. The Doppler sample volume was placed just distal to the pulmonary valve and the pulmonary artery flow pattern was recorded. The pattern was frozen on the screen and the time intervals were measured using the incorporated Doppler measurement calipers; a print was then taken. TPV was measured as the time interval between the systolic Doppler waveform leaving the baseline and reaching its peak velocity. RVET was the time interval between the systolic waveform leaving and returning to the baseline. A mean of three systolic waveforms was taken. A second observer, who was unaware of the initial results, repeated the measurements on 50 of the Doppler prints using a computer digitiser system. The correlation coefficient between the 'on line' and repeat measurements was 0·92.

The ductus arteriosus was then visualised from the high left parasternal 'ductal' view.[8] The Doppler sample volume was placed in the pulmonary end of the duct and the flow pattern recorded. The duct was designated closed when it could not be seen, and no flow could be detected on a pulsed Doppler search of the pulmonary end of the duct. Finally the foramen ovale was visualised from the subcostal four chamber view, and when possible the flow pattern was recorded.

The infants were initially studied in the first 12 hours after birth; studies were then repeated at 24 hour intervals until a steady state was reached.

Statistical analysis was by the unpaired Student's *t* test and the χ^2 test; p values of $<0·05$ were accepted as significant.

Thirty seven infants were studied, 18 born at full term and 19 preterm (table 1). All the preterm infants had either minimal or no extra oxygen requirements; two were initially placed in low concentrations of oxygen (<30%) but

Table 1 Comparability of the groups. Figures are given as mean (range)

	Infants born at full term (n=18)	*Preterm infants (n=19)*
Gestational age (weeks)	39·8 (38–42)	31·8 (28–34)*
Birth weight (g)	3300 (2650–4030)	1610 (950–2050)

*Seven were between 28 and 31 weeks' gestation.

both were breathing air within 24 hours. Consent to the study was obtained from all the mothers, and it was approved by the central Oxford research ethics committee.

Results

The TPV:RVET ratio rose in all infants in the early postnatal period. To compare the rate of change between those born at full term and those who were preterm the measurements were divided into postnatal age groups (table 2). For each age group the mean (SD) TPV:RVET ratio was plotted at the mean measurement time (figure). In the infants born at full term the TPV:RVET ratio rose rapidly over the first 30 hours of life, with only a minimal change after that. The rise in the TPV:RVET ratio was slower in the preterm infants, with the rise levelling out after about 60 hours. Within the first 6 hours, the mean TPV:RVET ratio was 0·23 (0·035) in the infants born at full term and 0·195 (0·042) in the preterm infants; the difference is not significant. In subsequent postnatal age groupings the ratio was significantly less in the preterm infants. Between 25 and 36 hours, the mean TPV:RVET ratio was 0·36 (0·037) in the infants born at full term and 0·29 (0·046) in the preterm infants, $p<0·001$. Between 73 and 96 hours there was no longer any significant difference, the mean being 0·38 (0·023) in those born at full term and 0·35 (0·045) in the preterm infants.

Table 2 Total number of studies in each age group

Age group (hours)	*Infants born at full term (n=18)*	*Preterm infants (n=19)*
0–6	8	10
7–14	9	8
15–24	0	6
25–36	18	13
37–48	2	6
49–72	17	18
73–96	10	13

Table 3 No (%) of studies in each age group that showed a closed ductus arteriosus. No infant had more than one study in each group. None of the differences are significant

Age group (hours)	*Infants born at full term (n=18)*	*Preterm infants (n=19)*
0–19	1/18 (5)	0/17
20–39	12/17 (71)	10/19 (53)
40–59	14/15 (93)	15/17 (88)
≥60	12/12 (100)	18/19 (95)

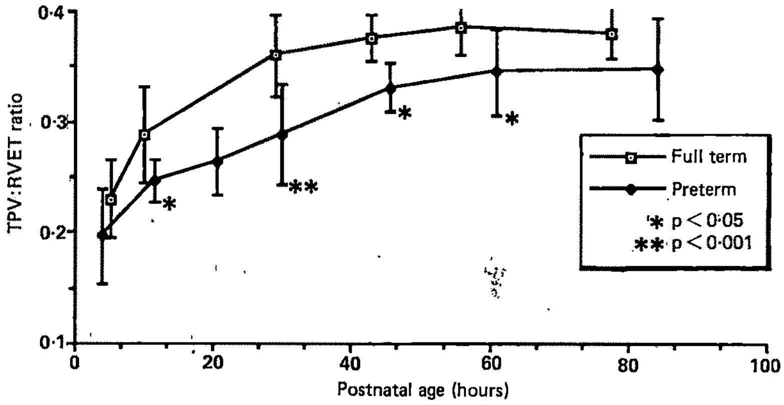
During the first 20 hours of life, the duct was closed in one of 18 studies in the infants born at full term and in none of 17 studies in preterm infants (table 3). Between 20 and 39 hours, 12 of 17 studies in infants born at full term and 10 of 19 studies in the preterm infants showed closed ducts; this difference is not significant. After this time most of the ducts were closed in both groups. The latest studies that showed a patent ductus were at 40 hours in an infant born at full term and at 72 hours in a preterm infant.

Apart from some of the studies during the first eight hours, the patterns of ductal flow were constant left to right throughout the cardiac cycle. Within the first eight hours, five of 10 studies in infants born at full term and five of nine studies in preterm infants showed bidirectional ductal flow. In these studies the flow was right to left in early right ventricular systole and left to right in late systole and diastole.

The Doppler pattern of flow through the foramen ovale was recorded in all the infants born at full term but, because of incompleteness of the early studies, in only seven of the preterm infants. The pattern of flow was predominantly left to right in all studies, flow was minimal or slightly right to left during ventricular diastole, and then became left to right increasing to a peak during atrial filling in ventricular systole. The velocity of this peak was usually less than 0·4 m/sec but in four of the infants born at full term peak velocities of up to 0·6 m/sec were seen.

Discussion

We found, using an indirect method of assessment, that pulmonary artery pressure in infants born at full term falls rapidly over the first 30



Comparison of the change in the time to peak velocity: right ventricular ejection time (TPV:RVET) ratio in the main pulmonary artery with postnatal age in healthy infants. For each age group values are shown as mean (SD).

hours of life. This is consistent with previous studies, both those using Doppler assessment[4–6] and those using direct measurement.[7] Emmanouilides *et al*, using catheters, showed a fall in pulmonary artery pressure from a mean of 40 mm Hg within the first 6 hours of life to a mean of 29 mm Hg between 24 and 36 hours.[7] This corresponds to the rise in the TPV:RVET ratio from 0·23 to 0·36 that we found. Our findings suggest, however, that the postnatal fall in pulmonary pressure is slower after preterm birth, even if there are no respiratory problems.

The development of pulmonary vasculature in later gestation has been well studied in fetal animals. Fetal lambs show a steady increase in pulmonary blood flow with a gradual fall in pulmonary vascular resistance during the second half of gestation.[9] Some of this change will be the result of the increase in cross sectional area of the vasculature of the developing fetal lung.[10] Prostaglandins seem to be important factors in the control and maintenance of in utero pulmonary vascular resistance and its subsequent postnatal fall. Prostaglandin synthesis in animal fetal lungs changes with advancing gestation; a predominance of constrictor prostaglandins (prostaglandin $F_{\alpha 2}$) in early gestation gives way to one of dilator prostaglandins (prostaglandin I_2, prostaglandin E_2) nearer full term.[10] After birth, high concentrations of prostaglandin I_2 are found in animal pulmonary veins suggesting that it may act as a mediator of the postnatal fall in pulmonary vascular resistance.[11] A combination of structural and hormonal immaturity may combine to produce a delayed fall in pulmonary artery pressure after preterm birth.

The pattern of flow through the ductus arteriosus was bidirectional in some of the early studies in both groups of infants. This pattern of shunting is seen when pulmonary artery pressure is equal to or slightly less than aortic pressure and thus is compatible with high early postnatal pulmonary artery pressures.[12] In the later studies, as pressures were falling, the pattern of flow was left to right throughout the cardiac cycle. Persistent ductal shunting is a well recognised problem for preterm infants with respiratory distress. Less ductal muscle mass,[13] decreased responsiveness to oxygen induced vasoconstriction,[14] and increased concentrations of dilator prostaglandin[15] have all been implicated in prolonged ductal patency. In preterm infants with no respiratory problems the ductus arteriosus closes within the first 3 to 4 days of life.[16] Our study, which included a larger number of less mature infants, confirms this finding. The latest study time showing a patent duct was 72 hours after birth in an infant of 31 weeks' gestation. Though preterm infants tended to close their ducts later, within the range of gestation that we studied prematurity without respiratory problems did not seem to prolong ductal shunting significantly.

The dominant direction of interatrial shunting was from left to right even in the early postnatal period, which is in keeping with the direct catheter studies that showed that pulmonary diastolic pressures fall earlier than systolic.[7] The foramen ovale does not completely seal for some time after birth, and our data suggest a degree of incompetence in most infants during the early postnatal period. The degree of that incompetence varies considerably among individual infants, four of the infants born at full term having appreciable atrial shunts. Using cross sectional echocardiography, Fukazawa *et al* found 24 out of 102 normal term infants had visible defects in the atrial septum, which had spontaneously closed in all but one of the infants by 1 year.[17] Thus it seems that the natural history of incompetence of the foramen ovale is for spontaneous closure.

Our results suggest that there are small but significant differences in the speed with which healthy preterm and term infants adapt to life outside the uterus. Though these differences are of physiological rather than clinical importance they do provide normal values from which we can go on to study preterm infants with respiratory disease as well as mature infants with persistent pulmonary hypertension.

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Neurodevelopmental outcome of transient neonatal intracerebral echodensities

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Abstract

The later neurodevelopmental progress of 15 babies who had neonatal periventricular echodensities or flares in the absence of any intraventricular bleeding or subsequent cystic degeneration was studied. At follow up four infants had neurological abnormalities, including spastic diplegia (n=2). These findings suggest that transient flares may represent mild periventricular leucomalacia with consequent mild neurological dysfunction.

The findings on cranial ultrasound, and the neurodevelopmental outcome, of preterm infants with cystic periventricular leucomalacia are well known. Although the pattern of evolution of the cysts that characterise periventricular leucomalacia is not clear, it is likely that the large or persistent (or both) periventricular echodensities or 'flares' that have been seen on ultrasound scans represent ischaemic lesions that subsequently undergo cystic degeneration. Infants with these lesions usually have moderate or severe neurological consequences. Although the clinical importance of smaller or transient echodensities, or both, is less well established, limited data suggest that they may also be associated with neurological dysfunction.

We describe the neurodevelopmental outcome in a group of 15 infants in whom isolated and transient intracerebral flares were seen on cranial ultrasound scans during the neonatal period. Our findings support those of previous studies in suggesting that these flares may represent mild periventricular leucomalacia.

Patients and methods

The cranial ultrasound scans of all newborn infants admitted to our regional neonatal intensive care unit during the four year period May 1984 to May 1988 were reviewed to identify those showing transient intracerebral echodensities. In this unit routine and serial scans are carried out on any infant whose gestational age is less than 34 weeks, any infant who is given ventilatory support irrespective of gestational age, and any infant who shows abnormal behaviour or neurological signs, or both. The scans were carried out in both coronal and parasagittal planes through the anterior fontanelle, and interpreted by a paediatric radiologist (REJL). Scans were done with a Hewlett-Packard 77020AR real time phased array sector scanner with a short focus 5 MHz probe. Infants were usually scanned within 24 hours of birth, and thereafter twice or thrice weekly until they were discharged home or transferred back to their referring hospital.

In this four year period one or more scans were carried out on a total of 727 infants, of whom 15 had transient echodensities or flares within the cerebral parenchyma in a periventricular distribution. A 'flare' was defined as an echodensity which was present in both coronal and parasagittal planes and which, irrespective of duration, size, or location resolved without the subsequent development of cysts, irregularly shaped ventricles, or other abnormal findings seen on ultrasound scan. None of the 15 infants had ultrasonic evidence of germinal matrix or intraventricular haemorrhage. Review of the clinical records and follow up (neurological examination and Denver developmental assessment corrected for gestational age) were undertaken by a single observer (REA).

Results

Details of the demographic, cranial ultrasound, and neurodevelopmental findings of the 15 infants are shown in the table. Of the six twins in this series, five shared a single placenta (cases 2–6) but in only case 2 was the placenta monochorionic. All the flares appeared within the first week of life. In 14 the parietal lobe(s) superolateral to the lateral ventricles were affected (bilateral in seven, and right sided in five). In the remaining infant (case 15) both caudate nuclei were affected. In eight infants there was complete resolution within seven days, while in four resolution was noted between 15 and 36 days after the onset. In the remaining three patients, no follow up scans were available; in one (case 13) the flares were unchanged six days after onset. These three infants were included in the study as the initial scan findings were identical to those of the rest of the group.

All the infants survived. The neurological examination was normal in 11 infants (73%). Two infants had spastic diplegia (one of which was pronounced and accompanied by a bilateral sensorineural hearing loss of 60 decibels (case 11), and one of which was minimal). Two other infants had abnormal and variable muscle tone (dystonia) and in one of these (case 13) hypotonia was the predominant finding; this child also had frequent and spontaneous shuddering spells. Two other infants (cases 8 and 9) had required surgical correction of bilateral squints. No child had developed epilepsy at the time of writing. Ten children (67%) had developed normally. Centiles for head circumference (at birth and at follow up) remained unchanged in

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Demographic, ultrasound, and neurodevelopmental findings in the 15 infants

Case No	Sex	Gestation (weeks)	Birth weight (g)	Singleton/ twin	Ventilatory support (days)	Onset to resolution of flares (days of life)	Age (months)	Neurological examination	Development
1	Male	29	890	Twin 2	33	2–9	16	Normal (but showed transient dystonia until 14 months old)	Normal
2	Female	29	1600	Twin 2	7	4–40	10	Normal	Normal
3	Female	31	1180	Twin 2	2	4–8	17	Normal	Gross motor and language delay
4	Male	31	1550	Twin 2	5	6–21	23	Spastic diplegia	Gross motor delay
5	Female	32	1840	Twin 2	7	5–28	9	Normal	Normal
6	Male	33	1875	Twin 1	0	1–6	35	Dystonia	Gross motor and language delay
7	Female	27	1180	Singleton	0	2–not recorded	26	Normal	Normal
8	Male	29	1510	Singleton	1	1–7	16	Normal	Normal
9	Female	30	1340	Singleton	0	2–6	17	Normal	Normal
10	Female	31	1620	Singleton	2	4–7	14	Normal	Normal
11	Male	33	1200	Singleton	10	4–35	14	Spastic diplegia	Gross motor and language delay
12	Female	33	2020	Singleton	0	2–8	28	Normal	Normal
13	Male	34	1560	Singleton	5	4–>10	16	Dystonia	Gross and fine motor delay
14	Male	34	2200	Singleton	2	1–5	41	Normal	Normal
15	Female	40	4115	Singleton	0	3–not recorded	16	Normal	Normal

10 infants. In five the centile increased by between 5 and 10%, but in only one (case 8) did this reach the 97th centile. The weight and height of this infant lay above the 97th centile, the neurodevelopmental status was normal, and cranial ultrasonography at follow up showed normal ventricles.

Discussion

Limited data have suggested that some periventricular echodensity is a common and transient finding, and therefore of no clinical importance.[1 2] There is no information, however, on the duration of 'transient', and therefore what constitutes 'normal' echodensity. Trounce *et al* arbitrarily defined prolonged, and presumably therefore clinically important, echodensities as lasting for two weeks or more and not becoming cystic.[3] De Vries *et al* observed that persistence of densities for more than 10 days was particularly associated with subsequent neurological problems.[4] In our study we included all infants with parenchymal echodensities or 'flares' of any duration, but excluded any infant who had evidence of germinal matrix or intraventricular haemorrhage in addition to flaring. Our reason for doing so was to try and correlate neurodevelopmental outcome with the duration of the flare alone. All our infants developed flares within the first week of life, a finding in agreement with that of Trounce *et al*.[3]

Several follow up studies have been reported that associate neurodevelopmental outcome with the presence of cystic periventricular leucomalacia.[5–9] The outcome of infants with flares or intracerebral echodensities that have not undergone cystic degeneration has been reported less frequently.[4 10 11] Of the 15 patients in our study 11 (73%) have a normal neurological examination; however, one of these 11 (case 3) is developmentally delayed. One infant, although normal at the time of this assessment, had shown abnormal muscle tone in the first year of life (transient dystonia). In two cases (2 and 5), flaring persisted for over three weeks; as their ages were only 9 and 10 months, respectively, however, these infants may yet develop neurological dysfunction. Four infants (all boys) had neurological abnormalities; in two the flares had persisted for over two weeks. In a further patient who had dystonia (case 13), flaring was unchanged six days after onset. It is therefore likely that the flares may have persisted for some considerable time.

McMenamin *et al*, in reporting 22 survivors of 32 infants with small intraparenchymal echodensities, found that complete resolution occurred in 19 while the remaining three developed small cysts.[10] Fourteen of the 22 infants (64%) were normal neurologically, and six demonstrated mild, and two moderate, deficits. All but one of their surviving pateints, however, also had germinal matrix or intraventricular haemorrhages, and there were no data on the duration of the echodensities. Data from the Hammersmith Hospital that identified periventricular echodensities in 59 infants, showed that there was a normal outcome in about half.[4] Four infants developed spastic diplegia and 24 transient dystonia. Infants with periventricular densities in association with germinal layer or intraventricular haemorrhages, or both, were also excluded from their study. The neurodevelopmental outcome of the infants with prolonged flares initially reported by Trounce *et al* indicated that such infants had a higher risk of neurological dysfunction,[3] but that the presence of prolonged flares was less accurate in predicting adverse outcome than for cystic periventricular leucomalacia.[11] The results of a further study also showed that persistent echodensities (lasting between two and four weeks) were associated with severe neurological handicap.[9]

Frequent and unprovoked episodes of shuddering were observed in one of the dystonic infants (case 13). Shuddering attacks are considered to be benign, unrelated to epilepsy, and a possible early clinical manifestation of essential tremor.[12] There is no evidence that they are associated with cerebral injury. The small number of patients in this study precludes a definitive conclusion about neurodevelopmental outcome with respect to the laterality of the flares. Thus those infants who had bilateral echodensities fared no better or worse than those with unilateral flares.

One third of the infants in this study were the second of twins (one of the six having a monochorionic placenta)—a relatively high proportion of the entire group. This is not necessarily unexpected, given that twin pregnancies (and particularly the second twin) are at increased risk of adverse peripartum events that may predispose to hypoxic or ischaemic brain damage, or both. It has also been suggested that a monochorionic placenta increases this risk even further.[13]

Our results show that neonatal transient intracerebral echodensities are not always benign. Thus all infants showing such echodensities should be carefully followed up and parents should be given a guarded prognosis about their neurological development.

The authors express their appreciation to the paediatricians of the Northern region for permission to study their patients.

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Birthweight ratio and outcome in preterm infants

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Abstract

The association between birthweight ratio and outcome was investigated in 429 infants born before 31 weeks' gestation. Birthweight ratio was calculated in each case as birth weight divided by mean birth weight for gestation (from reference data). It was shown that a given ratio corresponded to the same birth centile across the gestational age range studied; a ratio of 0·8 corresponding to the 10th centile. There was a linear relationship between birthweight ratio and requirement for mechanical ventilation and postneonatal mortality. Birthweight ratio was also strongly and linearly related to body weight, length, and head circumference at 18 months' corrected age. Overall, there was no association between this ratio and neurodevelopmental outcome to 18 months. However, the subgroup with the largest weights for gestation (birthweight ratio ≥1·1), had significantly higher language subscores than all the other children.

Our data show that conventional dichotomous categorisation of preterm infants into small or appropriate for gestation is inadequate when exploring the association between size for gestation and outcome.

Babies born small for gestation have been regarded as a special and high risk group. It has been suggested that such infants may have an increased rate of mortality or later neurodevelopmental impairment and reduced longer term growth potential.[1–5] It is well recognised that very preterm infants also constitute a high risk group for an impaired outcome. There is conflicting information, however, on the outlook for preterm infants in relation to their size for gestation at birth.[6–11] Most studies have been small and many have either matched for birth weight or compared small and appropriate for gestational age infants within a cohort which is defined by an upper birthweight criterion, thus excluding the larger infants at some gestational ages. Furthermore, the customary approach of comparing outcomes in 'small for gestation' with 'appropriate for gestation' infants, when the latter group comprises all from the 10th through the 99th centile, has never been satisfactorily justified.

Brooke *et al*, in a study of factors influencing birth weight, derived a continuous variable to define size for gestation, termed the 'birthweight ratio'.[12] This ratio was calculated as the infant's birth weight divided by reference median birth weight for the baby's gestation. Thus a birthweight ratio of 1 would signify that a baby's weight was on the 50th centile, whereas with a ratio of 0·80 a baby would be 20% lighter than the median. For birthweight ratio to be used for prognostic purposes in preterm infants of widely varying gestations, however, it would be important that a given value of this ratio corresponded to the same birth centile at different gestations. Having confirmed that this was the case (see below), birthweight ratio was calculated for 429 infants of less than 31 weeks' gestation, and data were examined for associations between birthweight ratio and outcome in the neonatal period and at 18 months' post term.

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Subjects and methods

The 429 infants in this study were from a larger five centre trial on feeding in babies weighing less than 1850 g at birth.[13] We report here on all enrolled infants born before 31 weeks of gestation, as above that gestation the larger infants (>1850 g) would have been excluded. Examination of records in all the centres confirmed that no infants of 30 weeks' gestation or less had a birth weight over 1850 g. Infants were enrolled in the trial within the first 48 hours of life; 53 eligible infants (without major congenital malformation and with median gestational age 26 weeks) died before enrolment.

Extensive social data were collected for each baby at parental interview. Social class was coded using the Registrar General's Classification,[14] with social class 3 subdivided into manual and non-manual. Maternal education was categorised as: 1, no educational qualifications; 2, less than four passes in the certificate of secondary education examination (CSE); 3, four or more CSE's or any 'O' level passes; 4, any 'A' level passes; and 5, degree or higher professional qualification. Birth rank was the birth order of the child in the living children of the family, with those from multiple births assigned equal rank. Details of neonatal course were recorded prospectively.

Birthweight ratio was calculated as the ratio of each infant's birth weight to a reference value of median birth weight for the baby's gestation. This reference value was taken from data, previously published,[15 16] for babies from the same cohort born after spontaneous onset of labour in the five centres. Infants born by elective caesarean section had been excluded from these reference statistics because their inclusion would have resulted in a group heavily biased by growth retarded infants who are often delivered interventively. The reference data were also used to calculate the correspondence between birth centile and birthweight ratio, and, as an important aspect of validation, to show the constancy of this correspondence across gestational age groups.

Surviving infants were invited for follow up examination at 18 months from expected date of delivery. Children from the four East Anglian centres (Cambridge, Ipswich, King's Lynn, and Norwich) were examined by RM; RP organised follow up in the fifth centre, Sheffield. An extensive history was taken for each child, followed by physical and neurological examination, using the method described by Amiel-Tison and Grenier.[17] Naked weight, length (to the next succeeding mm using a horizontal stadiometer), and occipitofrontal head circumference (to the next succeeding mm using paper tape) were measured in each case. Several developmental tests were administered; Bayley mental and motor scales,[18] Vineland social maturity scale,[19] and the questionnaire based academic scale of developmental profile II.[20] Scores for each child were calculated using the child's age from expected date of delivery (that is, corrected for prematurity). Mental and psychomotor development indices were derived from the results on the Bayley scales, social quotient from the Vineland test, and IQ equivalent was calculated using the academic scale of developmental profile II.

Student's *t* and χ^2 were used for univariate analyses. Regression analysis was used where adjustment for confounding factors was required (linear for continuous and logistic for dichotomous dependent variables).

Results

In order to explore whether the correspondence of birth centile and birthweight ratio was similar across gestational age groups, the ratio corresponding to 3rd, 10th, 90th, and 97th centiles was calculated for each gestation from the smoothed reference data referred to above.[15] (For example, the ratio corresponding to the 10th centile at 28 weeks=weight on the 10th centile at 28 weeks divided by median weight at 28 weeks.) The ratio was remarkably constant, at gestations from 25 to 30 weeks, for any of the four centiles studied. Thus for the 3rd centile, the ratio only varied between 0·70 and 0·72 across gestational age groups. Corresponding values for the 10th centile were 0·80–0·81; the 90th centile: 1·19–1·20, and the 97th centile 1·28–1·30. The figures are consistent with a birthweight ratio that is normally distributed with a constant coefficient of variation across gestations of 15%.

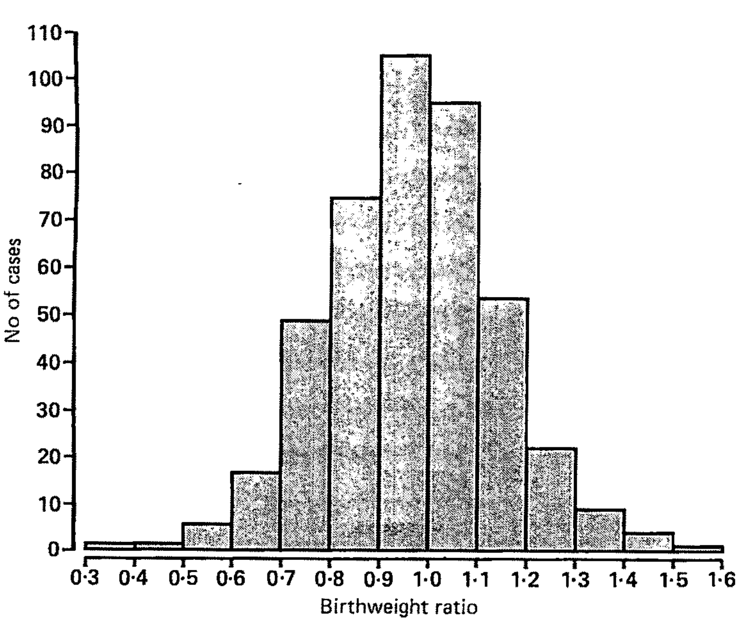

Figure 1 Distribution of birthweight ratios within the study cohort.

The distribution of birthweight ratio within the 429 infants in this cohort is shown in fig 1. The distribution (range 0·39 to 1·51) shows the expected left shift, reflecting the increased proportion of small for gestation infants, largely delivered interventively, found among those born preterm.[15]

The association between an infant's birthweight ratio and those social and demographic factors known to have an important influence on later developmental status[21] was investigated. Birthweight ratio was not significantly related to social class, birth rank, or sex of the child or to maternal age, education, or marital status.

Length of gestation was investigated for association with birthweight ratio. Infants were divided into the five birthweight ratio categories: <0·8, 0·8–0·9, 0·9–1, 1–1·1, and ≥1·1, giving groups of broadly comparable size. Infants in the lowest ratio group (<0·8) were all less than the 10th centile and would be classified in many studies as small for gestation. There were no significant differences in mean gestation between the five birthweight ratio categories (table 1), though there were variations (range 28·1–28·8 weeks). In subsequent analyses outcome data are presented by birthweight ratio category (as above). In addition, regression analysis has been used to relate birthweight ratio, as a continuous variable, to each outcome, while adjusting for gestation, to ensure that the small differences in gestation between birthweight ratio categories did not confound the analysis.

No association was found between birthweight ratio category and mortality in the first 28 days (table 1). Infants were enrolled in this study within the first 48 hours, however, so those dying soon after birth would have been excluded. There was evidence, however, of higher postneonatal mortality with decreasing birthweight ratio group. This association was highly significant when logistic regression analysis was used to relate ratio (as a continuous variable) to postneonatal mortality, adjusting for gestational age (p<0·001).

Birthweight ratio was related to the duration of mechanical ventilation. For this analysis data from the 45 babies who died in the first 28 days were excluded, as early death would have curtailed the period of respiratory illness. There were 384 survivors of the neonatal period, of whom 200 were boys. Table 2 shows the proportion of infants in each ratio group requiring mechanical ventilation in the first 24 hours, for

Table 1 Birthweight ratio and mortality

	Birthweight ratio groups				
	<0·8 (n=71)	*0·8 to <0·9 (n=74)*	*0·9 to <1·0 (n=104)*	*1·0 to <1·1 (n=94)*	*≥1·1 (n=86)*
Mean (SE) gestation (weeks)	28·8 (0·15)	28·3 (0·17)	28·1 (0·15)	28·1 (0·14)	28·5 (0·15)
No (% occurrence) dying in first 28 days*	4 (6)	8 (11)	15 (14)	9 (10)	9 (10)
No (% occurrence) dying postneonatally	8 (11)	9 (12)	6 (6)	3 (3)	0 (0)***

*Infants were enrolled in this study within 48 hours of birth, therefore very early neonatal deaths are not included.
***p<0·001 for birthweight ratio (as a continuous variable) regressed against whether each subject died in the postneonatal period (adjusting for gestation).

Table 2 Birthweight ratio and requirement for mechanical ventilation (infants dying in the neonatal period are excluded from these analyses)

	Birthweight ratio groups				
	<0·8 (n=67)	*0·8 to <0·9 (n=66)*	*0·9 to <1·0 (n=89)*	*1·0 to <1·1 (n=85)*	*≥1·1 (n=77)*
No (%) ventilated in first 24 hours	54 (81)	57 (86)	71 (80)	64 (75)	48 (62)***
No (%) ventilated >7 days	26 (39)	23 (35)	26 (29)	25 (29)	17 (22)***
No (%) ventilated >14 days	15 (22)	15 (23)	16 (18)	14 (16)	4 (5)***
No (%) ventilated >28 days	7 (10)	7 (11)	9 (10)	7 (8)	1 (1)**

**p<0·005, ***p<0·0005, for birthweight ratio (as a continuous variable) regressed against the need for ventilation in the first 24 hours and for over 7, 14, or 28 days (adjusting for gestation).

more than seven days, more than 14 days, and more than 28 days. There was a significant association between ratio group and need for mechanical ventilation at each of these cutoff values (respectively: p<0·025, p<0·001, p<0·025, and p<0·001, using χ^2 test for trend). These associations were confirmed using logistic regression analysis, adjusting for gestational age (respectively: p<0·0005, p<0·0005, p<0·0005, and p<0·005).

Altogether 329/358 (92%) of surviving infants were examined at 18 months post term. Mean weight, length, and head circumference at 18 months is shown for each ratio group in table 3. There was a progressive increase in each of these growth measures with increasing birthweight ratio (p values for trend were respectively <0·0001, <0·0001, and <0·002). While data are shown for both sexes combined, very similar trends were seen when data from boys and girls were analysed separately (as expected, there was a consistent trend to higher values for body weight, length, and head circumference in boys). Although there were no significant differences in sex distribution between ratio groups, there were variations that could have confounded the analyses above. Regression models were therefore used to explore the association between anthropometry at 18 months (dependent variable) and birthweight ratio (as a continuous variable), after adjusting for the sex of the child as well as gestation. There were highly significant associations between birthweight ratio and weight and length (both p<0·0001) and head circumference (p<0·003) at 18 months' post term.

It is of interest that of the 329 children seen at follow up, 143 (43%) had birthweight ratios of 1·0 or more, indicating that their weight at birth was on or above the 50th centile, whereas at 18 months, only 75 (23%) had weights on or above the 50th centile, using the Whitehead standards (unpublished data).[22]

The number of children in each ratio group diagnosed as having neurological impairment (including those with little or no handicap) is shown in table 4. There were no significant differences between the groups. If these impaired children are combined with those whose Bayley mental development index was less than 70, to form a group we have described here as 'neurodevelopmentally impaired' there was again no significant difference between birthweight ratio groups. There was no significant association between ratio group and either mean Bayley mental score, Bayley motor score (table 4), IQ equivalent or social quotient, whether or not children with neurological impairment were included in the analysis. Regression analysis, adjusting for gestational age, sex and birth rank of the child, social class, and maternal age and education also failed to show any association

*Table 3 Birthweight ratio and growth to 18 months post term**

	Birthweight ratio groups				
	<0·8	*0·8 to <0·9*	*0·9 to <1·0*	*1·0 to <1·1*	*≥1·1*
No followed up	56	54	76	77	66
Mean (SD) body weight (kg)	9·3 (1·2)	9·9 (1·3)	10·2 (1·1)	10·2 (1·2)	10·7 (1·3)***
Mean (SD) body length (cm)	77·7 (3·5)	79·0 (3·3)	79·7 (3·3)	80·1 (3·2)	80·7 (3·0)***
Mean (SD) head circumference (cm)	47·4 (1·6)	47·9 (1·9)	48·2 (1·7)	48·2 (1·5)	48·3 (1·5)**

*For reference the 50th centile (sexes combined) for weight, length, and head circumference at 18 months is: 10·7 kg, 80·3 cm, 48·7 cm; and the 10th centile: 9·5 kg, 76·8 cm, 46·8 cm.
**p<0·003, ***p<0·0001, for birthweight ratio (as a continuous variable) regressed against either weight, length, or head circumference, adjusting for sex and gestation.

Table 4 Birthweight ratio and neurodevelopmental outcome at 18 months

	Birthweight ratio groups				
	<0·8	*0·8 to <0·9*	*0·9 to <1·0*	*1·0 to <1·1*	*≥1·1*
No followed up	56	54	76	77	66
No (%) with neurological impairment	5 (9)	4 (7)	12 (16)	8 (10)	9 (14)
No (%) with neurodevelopmental impairment*	5 (9)	8 (15)	16 (21)	14 (18)	12 (18)
Mean (SD) Bayley mental development index	100 (17)	98 (20)	99 (17)	99 (21)	103 (20)
Mean (SD) Bayley motor development index	91 (19)	88 (16)	90 (19)	86 (20)	88 (17)
Mean (SD) language subscore as quotient	96 (18)	93 (18)	92 (20)	96 (19)	101 (19)**

*Neurodevelopmental impairment was diagnosed as neurological impairment or mental index <70.
**$p<0\cdot01$, for language subscore in infants with birthweight ratios 1·1 or above compared with all infants with a ratio <1·1.

between birthweight ratio and these measures of developmental outcome.

When the language score was calculated (using the Kent scoring adaptation of the Bayley mental scale[23]), however, those in the highest ratio group (ratio 1·1 or above) had a significantly higher mean score than the other groups combined ($p<0\cdot01$). Conversely, those in the lowest ratio group (infants who would usually be categorised as small for gestation) did not differ from the other groups combined. Regression analyses (adjusted as above) confirmed that those children in the highest ratio group (≥1·1) had significantly higher language scores than either those in all other groups combined ($p<0\cdot005$) or in a 'control group' of babies with ratios of 0·9 to 1·1, in the middle of the normal range ($p<0\cdot05$). Conversely, similar regression analyses confirmed that there was no difference in language score for children in the lowest ratio group compared with those in the other groups combined (ratio 0·8 and above), or those with ratios 0·9–1·1.

Discussion

Our study has shown that weight for gestation is a major factor relating to short term morbidity and postneonatal mortality and to longer term growth and language development. Nevertheless, as we argue below, the data presented show that in outcome studies of preterm infants, the conventional approach of dividing infants arbitrarily into 'small' (<10th centile) and 'appropriate' (≥10th centile) for gestation may be quite inadequate to describe the influence of birth weight relative to gestation.

Distribution of birthweight ratios within this group of 429 infants born before 31 weeks of gestation differed from that expected in an unselected population. Many infants in this group were delivered interventively because of intrauterine growth retardation or maternal hypertension, reflecting the substantially increased proportion of small for gestational age infants seen among those undergoing modern neonatal intensive care.[15]

We observed that with increasing birthweight ratio there was a progressive and significant decrease in the need for mechanical ventilation, when the latter was expressed either as a continuous variable (days of ventilation) or as a series of dichotomous variables (proportion of subjects requiring ventilation in the first 24 hours and beyond either 7, 14, or 28 days). Our findings indicate therefore that the incidence of chronic lung disease diminishes with increasing body size for gestational age. As an example fig 2 shows the estimated proportion of subjects born at 27 weeks' gestation requiring ventilation in each of the five, approximately equally sized birthweight ratio categories. Estimates are based on absolute odds calculated from logistic regression analyses. Data shown in this figure emphasise that the association between size for gestation and requirement for mechanical ventilation is linear across the entire range of birthweight ratio categories. Thus to compare infants in the lowest ratio category (<0·8, <10th centile) with the remainder would be arbitrary and would not result in adequate biological description of the data.

Our data on requirement for mechanical ventilation in surviving infants would lead us to expect that neonatal mortality might be higher with decreasing ratio. We were, however, unable to show the expected increase in mortality in the first 28 days with lower birthweight ratio, though we might have missed such a relationship as a result of recruiting most infants between 24 and 48 hours after birth, before which there might have been a selective loss of infants in the lowest birthweight ratio categories. Nevertheless, for postneonatal mortality (after 28 days), there was indeed a pronounced linear decline with increasing birthweight ratio. It is clear from the mortality data in table 1 that it would have been inappropriate to have categorised infants into 'small' and 'appropriate' for gestational age.

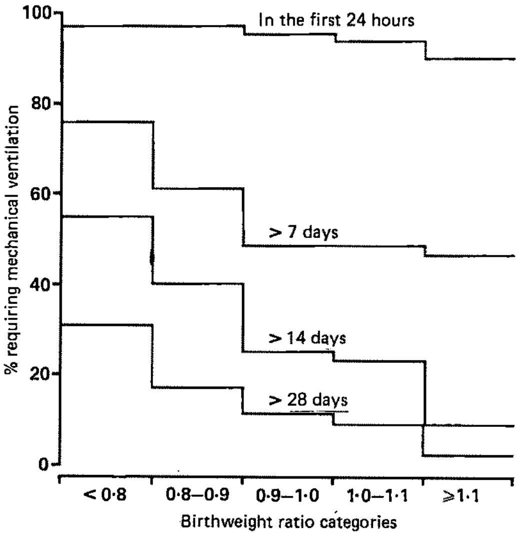

Figure 2 The estimated proportion of 27 week infants in each birthweight category requiring mechanical ventilation in the first 24 hours and beyond 7, 14, and 28 days. (Calculated from absolute odds based on logistic regression analysis.)

In many studies on small for gestational age infants born at term, long term growth potential has been shown to be impaired.[4,5] Corresponding data for growth retarded infants born preterm are more limited.[24,25] In this study birthweight ratio was a powerful predictor of weight, length, and head circumference, even after adjustment for the potentially confounding factors. The association between growth and birthweight ratio was again linear across all birthweight ratio categories, the infants with the highest ratios exhibiting the largest birth weight, length, and head circumference values. As a whole the infants investigated here were growth retarded at 18 months. The proportion of infants above the 50th centile for birth weight was 43%; whereas only 23% of infants were over the 50th centile for weight at 18 months.[22] Indeed at 18 months even the infants from the highest birthweight ratio category (ratio $\geq 1\cdot 1$), had mean body weight and length close to the 50th centile and mean head circumference below the 50th centile (sexes combined). Thus the larger the birthweight ratio, the greater the chance that the infant had attained average body size at 18 months. A dichotomous categorisation of infants into those above or below the 10th centile, would not have been adequate to demonstrate these findings.

Some studies have indicated that preterm small for gestational age infants have reduced developmental performance in childhood.[6,10,11] We were unable to confirm this using a battery of developmental tests, including Bayley mental and psychomotor scales, the Vineland social maturity and the academic scale of developmental profile II. Interestingly, only the language score (from the Kent subscores of the Bayley mental scale) showed an association with size for gestation and in this instance it was only the infants with the largest birthweight ratios ($\geq 1\cdot 1$) that were advantaged compared with the others. Planned follow up examinations in later childhood will investigate whether birthweight ratio is associated with differences in later IQ or learning performance.

Usher and McLean (RH Usher, FH McLean, unpublished data) have suggested that infants who are large for gestational age and born at term are disadvantaged in terms of developmental outcome; this, however, might relate to the strong positive association between maternal diabetes and birth weight. In contrast, in this study on very preterm infants, it was the infants with the largest body size for gestation who were significantly advantaged in short and long term outcome. Nevertheless, the association between birth weight and outcome in more mature preterm and term infants needs to be re-explored using birthweight ratio as a prognostic index. Results would be broadly similar if birthweight centile groups (<10th, 10th–25th, etc) were used, though for the researcher a continuous variable like birthweight ratio has advantages over a categorical one like centile strata. Furthermore, birthweight ratio is a more direct measure of birth weight for gestation.

In conclusion, our findings show that with increasing birth weight for gestation, preterm infants had a progressively better prognosis in terms of their need for respiratory support, or death in the early months, and had a progressively improved long term growth performance. Our observations suggest that an arbitrary dichotomous categorisation into small or appropriate for gestation groups is inadequate as a prognostic index in preterm infants.

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Pupillary diameter and reaction to light in preterm neonates

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Abstract

The pupil controls the amount of light entering the eye. We have examined both the time course for the development of the light reflex and pupil diameter before and after this event in 50 neonates. The pupillary light reflex was absent in all neonates of less than 30 weeks' gestational age, it gradually developed from this age and by 35 weeks it was present in all. Several possible mechanisms that may explain the onset of this reflex are considered. Before the onset of the pupil reflex mean horizontal pupillary diameter was 3·46 mm compared with 3·02 mm after the reflex development. We have estimated the amount of light reaching the retina (retinal irradiance) for preterm neonates and although it is less than that known to cause retinal damage in animals, further research is necessary to determine whether early light exposure has an adverse or beneficial effect on the immature visual system.

The pupil regulates the amount of light entering the eye by reflex dilatation or constriction in response to changes in the illumination. Few studies have been performed that have explored the development of the light reflex in neonates. In 1966 Robinson investigated the development of 20 types of reflexes in an attempt to produce an assessment that would be useful to the paediatrician interested in distinguishing between preterm and small for date neonates.[1] Of the reflexes examined he found that the presence of the pupillary light reflex was one of five that were highly correlated with gestational age. Finnström designed a study to assess the use of reflex testing as part of a gestational age assessment.[2] Babies of less than 29 weeks' gestational age (n=4) had no pupillary response to light, but by 31 weeks' gestational age the reflex appeared to be present, although sluggish in all. When the presence of the pupillary light reflex was examined as a function of gestation, he found a correlation coefficient of only 0·24, although he accepted that this may have been due to the small number (n=6) of infants of less than 32 weeks' gestation in this study. In both of these studies the numbers of babies of less than 29 weeks' gestational age were small and their gestational ages were not given. This indicates the need to examine the pupil of the very immature infant: to date the pupil diameter of the preterm neonate has been mentioned on two occasions only and divergent values of 1·5 mm[3] and 2·9 mm[4] have been quoted. We therefore decided to study pupillary diameter and light reflex prospectively, and we report, first, the time course of the development of the pupillary light reflex and second,pupil diameter in infants with and without pupillary constriction to light.

Subjects and methods

SUBJECTS

Preterm babies with gestational age less than 37 weeks (WHO classification) admitted to the neonatal unit of the Leicester Royal Infirmary between February and July 1988 were enrolled in the investigation of pupillary diameter. Those who had no light reflex at the first examination were enrolled in the longitudinal study of reflex development. But for the study of pupil diameter, infants with a light reflex on the initial examination were also included. Postmenstrual age was defined as the gestational age, as assessed by the paediatrician at birth, plus the postnatal age. Ethical approval for the study was granted by the Leicestershire Area Health Authority. Full details of the infants are included in the table.

APPARATUS

The Medical-Nikkor Auto 200 mm f/5·6 lens was designed for taking close up photographs of known reproduction ratio of 1:15, use of an auxillary lens can alter this ratio to 1:1. As the lens is fixed-focus, focusing is achieved simply by pointing the camera at the eye and moving it back and forth until the eye is in sharp focus. The lens to subject distance was 221 mm using this combination of lenses and the subject field was 24 by 36 mm.

The lens was attached to a Nikkon FE camera back, and the power for the ring flash was produced by the supplied AC converter. The flash illumination was reduced to one fourth of

Details of subjects studied

Gestational (weeks)	*No of infants*		*Median (range) birth weight (g)*
	Total	*With pupils reacting to light*	
26	4	0	790 (760–820)
27	6	1*	750 (760–880)
28	4	0	855 (650–980)
29	6	0	1045 (1030–1060)
30	4	2	1095 (1030–1420)
31	4	4	1370 (1020–2121)
32	6	6	1570 (1520–1620)
33	6	5	1645 (1580–1700)
34	4	2	1750 (1680–2200)
>34	6	6	1990 (1520–2500)

*In this infant a slight, slow reaction to light was noted in the first examination. However, no response to light was noted in either of the next two examinations, made at weekly intervals.

its full value at higher reproduction ratios. The output of the ring flash was filtered using a combination of EE and 30 CC Blue filters (Kodak Wratten) which effectively blocks 99% of the ultraviolet light emitted by the flash. A flash may be used as the latency of the pupillary light reflex is always longer than the duration of the film exposure, thus the photograph will accurately record the size of the non-constricted pupil.[5]

Fast film (Kodak TMX Professional, ASA 400) was loaded to reduce the duration of exposure to a minimum. An aperture of f 32 was used, which in combination with the ×1 auxillary lens produces a depth of field of ±1·86 mm, thus the eye was only in focus when the lens to eye distance was in the range 219·14 to 222·86 mm. Similarly, the lens system could only produce clear images of the pupil if there was a 90° angle between the iris plane and the lens thus the camera must be 'on-axis' (with a tolerance of ±2°).

PROTOCOL

The babies were examined at weekly intervals while on the neonatal unit commencing in all during the first week of life. Eyelids were opened manually or in the case of very immature infants an eyelid speculum was used after instillation of topical anaesthesia oxybuprocaine hydrochloride 0·4%.

Photographs were taken usually first of the left eye; the presence of the pupillary reflex tested using the light from a direct ophthalmoscope (6W) for a minimum duration of five seconds. Iris colour was noted. Similar records were then made for the right eye after a minimum five minute interval.

ANALYSIS OF RESULTS

Horizontal and vertical pupil diameters were measured directly from the film negatives using a magnified graticule scale. Any image in which either the pupil was not clearly visible or the image of the ring flash was not in the centre of the pupil were discarded. Diameters were measured both by a naive observer and by one of the experimenters with good interobserver agreement being noted (r=0·93).

STATISTICAL ANALYSIS

Light reflex: methods of analysis and presentation

The results were grouped into six postmenstrual age categories. In the first category (<27 weeks), the pupil response to light was invariably absent while in the last category (>34 weeks) this was always present. For each period responses were included if the infant was born in or before that period. This has the advantage of combining 'longitudinal' study data and transverse studies.

Pupil diameter: methods of analysis and of presentation

Results were grouped both by postmenstrual age and by the presence or absence of the pupillary light reflex. The correlation coefficient between horizontal and vertical pupil diameters was calculated. Mean pupil diameters were derived from the weekly measurements both for neonates with and without the light reflex. These were then compared using an independent samples t test.

Results

Fifty premature neonates were studied, approximately balanced for sex. In addition 12 babies born before the onset of the pupil response died before the conclusion of the study and have therefore not been included in the analysis. All neonates were observed initially during their first postnatal week. Those infants who did not have a pupil response at first examination were enrolled in the longitudinal study of pupillary size and reflex development.

The relation between postmenstrual age and the presence of the pupillary light reflex is apparent in fig 1. In only one baby of less than 30 weeks' postmenstrual age was there a response to light, and in this infant the constriction was both very slow and slight. As there was no pupillary response to light in the next two examinations we concluded that our original observation was incorrect and this result was subsequently excluded from the analysis. No neonate of less than 30 weeks' postmenstrual age had a light reflex; by 34 weeks 86% (18/21) of all infants examined responded to an increase in illumination with a pupillary constriction and by 35 weeks this light reflex was present in all.

The presence of the pupillary reflex appears to be related to gestational age. The number of neonates who had a pupillary response to light at the initial examination are shown in the table.

When the speed of pupil reaction was taken into account the developmental nature of the light reflex becomes more obvious (fig 2). The changeover from a slow, minimal reaction to the characteristic brisk pupillary response occurred between 31 to 32 weeks' postmenstrual age.

It is of interest to look at the possible effect early exposure may have on the maturation of

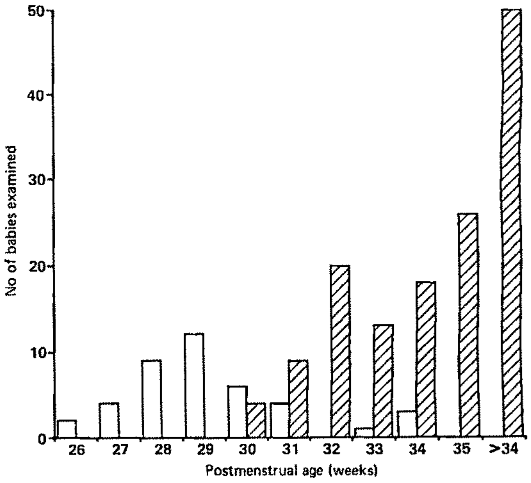

Figure 1 Presence of a pupillary constriction to light for neonates. Each bar represents the number of babies born at that gestation or earlier, with either no reaction (□) or a pupillary constriction to light (▨).

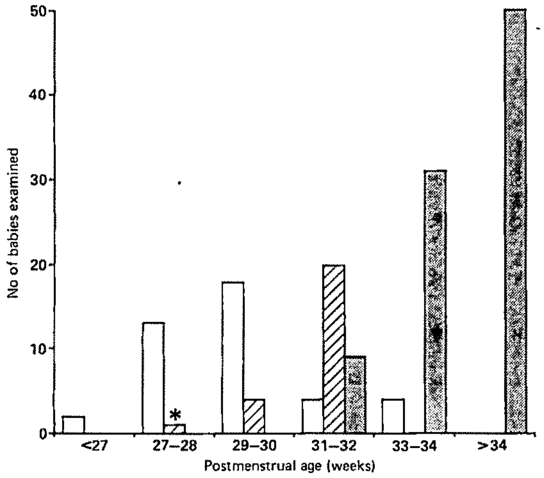

Figure 2 Speeds of pupillary constriction for neonates are grouped by postmenstrual age. Each bar represents the number of babies examined with no response (☐); a slow reaction (▨) or a normal brisk pupillary constriction to light (■). The asterisk indicates a baby who had a reflex constriction at 27 weeks but was subsequently found to have unreactive pupils when next examined one week and two weeks later.

this reflex. Following infants longitudinally the very immature neonates (≤27 weeks' gestational age) appeared to develop the pupillary light response at an earlier postmenstrual age than babies born at a later gestation. All babies whose pupils constricted to light at 30 weeks were born at ≤27 weeks' gestational age. Thus there is some evidence that early birth hastens the onset of the pupil response but as the number of infants born at this age was small (n=4) this trend could not be examined statistically.

Mean (SD) horizontal and vertical pupil diameters for all infants studied, calculated from the weekly measurements, were 3·14 (0·07) and 3·14 (0·07) respectively. These values include those obtained from infants who had no reflex response to light. As one of the functions of the pupil is to control the amount of light entering the eye it was decided to examine the two subpopulations separately. For babies with no pupillary light reflex (76 weekly measurements) mean horizontal and vertical pupil diameters were 3·46 (0·78) and 3·49 (0·72) mm respectively. While the mean pupil diameters for babies whose pupils reacted to light were 3·02 (0·84) and 3·00 (0·85) mm for the horizontal and vertical diameters respectively (n=200 weekly measurements). When compared using an independent samples *t* test, infants with no light response were found to have significantly larger pupils than those with a reflex pupillary constriction to light ($p<0·002$ and $p<0·006$ for the vertical and horizontal diameters respectively). Good interobserver correlation was found ($r=0·93$).

The colour of the iris was noted as part of the ocular examination. All neonates of less than 34 weeks' postmenstrual age had grey irides, with other colours developing from this time onwards.

Discussion

This study consists of two major components: first, the development of the pupillary light reflex and second, pupil size under standard neonatal unit illumination (mean 512 lux; range 395–628 lux) before and after the onset of this reflex.

Our observation that the pupillary light reflex was almost invariably absent in neonates of less than 30 weeks differs from Robinson who stated that the pupillary response to light is present in some babies of 29 weeks' gestation.[1] In the latter study, however, very few neonates of less than 30 weeks' gestational age were examined (n=7) compared with our study (n=21).

The low correlation coefficient between gestation and presence of the pupillary light reflex found by Finnström may also have been due to the small number (n=6) of infants of less than 32 weeks' gestation in the study.[2]

The pathway for the light reflex is subcortical. Thomson suggests that pupillary fibres originate from the retinal photoreceptors, thence to the retinal ganglion cell and the axon of the optic nerve.[6] These fibres are either very close to or are the same as those involved with visual acuity, as damage to the optic nerve is linked to defects in visual fields with disturbance of pupil responses. They leave the optic tract before the lateral geniculate nucleus to synapse in the pretectal region of the midbrain. Impulses are then relayed by crossed fibres through the posterior commissure to the opposite Edinger-Westphal nucleus of the oculomotor nerve. In addition, some fibres pass ventrally to the ipsilateral oculomotor nucleus. The efferent pathway is along the third nerve, via small fibres lying centrally within this nerve, which pass to the ciliary ganglion within the extraocular muscle cone in the orbit. Postganglionic fibres pass via the short ciliary nerves to innervate the sphincter muscle of the iris and other ocular structures.

It is now pertinent to consider some possible mechanism(s) that may account for the onset of the pupillary reflex to light. These include the functional immaturity of the retinal photoreceptors, iris musculature, or neurotransmitters. Other factors to be considered are myelination of the reflex pathway and anatomical restraint provided by the tunica vasculosa lentis. As mentioned the receptors for this response are probably those involved with vision.[6] That photoreceptor immaturity could explain the absence of the pupil response cannot be excluded. Retinal cones are present by 22 weeks' gestation,[7] and although they are not morphologically mature for about four years,[8] it is clearly not possible to correlate accurately morphology and function. It is relevant to note that the blink to light, a reflex dependent on functioning photoreceptors, is present in all babies ≥26 weeks' postmenstrual age.[1]

Magoon and Robb have detected traces of myelin in neonates of between 28 and 32 weeks' gestation near the optic chiasm[9]; however, the process of myelination not being completed in the anterior visual pathway for at least two years is probably too slow to account for the rapid onset of the pupil response noted in this study (fig 2). Anatomical restraint by the transient tunica vasculosa lentis and possible immaturity of the muscles of the iris need also be considered. It has been suggested that the transient tunica vasculosa lentis may hinder the contraction of the sphincter pupillae muscle thus preventing reflex constriction.[1] Tunica vasculosa lentis regresses between 28 and 34 weeks' gestation.[10] However, no anatomical evidence has been found to suggest that this membrane is linked to the iris. Indeed, very immature neonates, born at less than 28 weeks' gestation, are screened before the regression of the transient tunica vasculosa lentis for retinopathy of prematurity and in this situation pupillary pharmacological dilatation can easily be achieved. Finally, the sudden onset of the pupillary reflex could reflect maturation of the iris musculature, either due to insufficient release of or the over efficient reuptake or breakdown of the transmitter (acetylcholine) within the synaptic cleft. Most previous attempts to gain some understanding of the degree of maturity of these muscles in preterm infants have examined the pharmacological effects of various drugs on the muscle dilator pupillae. Lind and associates examined the effects of sympathomimetic amines on the pupil diameter of preterm neonates 28 to 40 weeks' gestational age.[11] Their results suggested that either nerve endings may not yet be able to release noradrenaline, noradrenaline is unavailable at this age, or that membrane immaturity may prevent uptake of both directly acting amines and of noradrenaline precursors.[12] They concluded that the end organ is capable of responding but the synthesis storage or release of neurotransmitter may be deficient.

The pupil modifies the amount of light entering the eye. Our results show that neonates who have yet to develop the pupillary light reflex have a larger pupil than those in whom this reflex is established (mean=3·46 mm compared with 3·02 mm). Only one study has measured pupil size in preterm neonates and this was mainly concerned with the effects of various mydriatic agents on diameter.[4] These authors measured pupil diameter by comparison with a series of circles of known diameter, obtaining an estimate of 2·9 mm at 35 weeks' postmenstrual age compared with our results: 3·02 mm at 35 weeks.

The potentially harmful effects of light on the developing visual system have recently been reviewed.[13] However, no measurements have been made of the retinal light dose actually received by an infant undergoing treatment on a neonatal unit. Retinal irradiance may be related to pupil diameter, transmission of light across the ocular media, brightness of the room, and the area of the retinal image.[3] [14] Hamer and coworkers have made the only previous attempt to calculate the neonatal retinal irradiance that was based on a pupillary diameter of 1·5 mm,[3] but according to our calculations this needs to be raised by a power of four. Further detailed consideration of this topic is beyond the scope of this paper.

We have shown that the pupil response to light develops between 30 and 31 weeks' postmenstrual age in preterm neonates. The pupil diameter in those infants with no reflex is larger than that in neonates who have developed the light reflex, thus the very immature baby will receive a larger retinal light dose than its older counterpart. We also recorded pupil diameters in a small sample (n=5) of babies whose eyes had been dilated using cyclopentolate (0·5% eye drops) before routine screening for retinopathy of prematurity. Mean pupil diameter after dilatation was 5·21 (0·08) mm. Therefore these infants will receive a proportionally greater retinal irradiance until the pharmacologically induced pupillary dilatation wears off.

The consequence of our findings on calculating the neonatal ocular light dose are considerable but further research is needed to determine whether early exposure to light has an adverse or indeed beneficial effect on any part of the immature visual system.

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Behavioural states in normal mature human fetuses

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Abstract

Behaviour was studied by real time ultrasound in a group of 80 low risk fetuses between 36 and 42 weeks' gestation. There was close linkage of fetal eye movements, somatic movements and heart rate pattern reflecting three different states. Quiescence (state 1F) was characterised by no eye movements, no somatic movements except for the occasional startle, and a fetal heart rate pattern with little baseline variability. There were two active states: state 2F characterised by continuous eye movements, frequent bursts of somatic movements, and wide baseline variability with accelerations with movement, and state 4F characterised by continuous eye movements with almost continuous somatic movements and a sustained tachycardia. These fulfilled criteria for three of four behavioural states previously described in human fetuses. Cycling of quiescent and active states occurred in 77 (96%) of the fetuses within 100 minutes of starting the recording. 2F was the commonest behavioural state, being seen 58% of the time. 1F was seen 30% of the time, and 4F 9% of the time. During the remaining 3% of observation time the behavioural state was indeterminate.

For several years we have known that healthy newborn infants normally manifest least five different behavioural states: quiet sleep (S1), active/rapid eye movement (REM) sleep (S2), quiet awake (S3), and active awake without crying (S4), and with crying (S5). These are characterised by recurring conditions of certain variables in stable association with one another. These variables include eye movements, heart rate pattern, breathing pattern, and electroencephalographic pattern and activity.[1] For practical reasons the criteria used for the states in the newborn are unsuitable for the fetus. Unlike the infant the fetus does not make breathing movements all the time, and opening and closing of the eyes cannot always be observed easily with ultrasound, nor can an electroencephalogram be recorded until after rupture of the membranes. In 1981, however, Bots *et al* validated the detection of fetal eye movements with ultrasound and it was this that made the study of fetal behavioural states possible.[2]

Using real time ultrasound recordings of fetal motility with simultaneous continuous recording of the fetal heart rate, Nijhuis *et al* analysed the temporal association of different types of somatic movements, eye movements, and heart rate patterns of fetuses of multiparous women that were between 32 and 40 weeks' gestation.[3 4] After 36 weeks they found distinct, recurring combinations of these variables that they considered analagous to the first four of the five behavioural states described in infants (S1 to S4), and they therefore designated them states 1F to 4F. Arduini *et al* have studied the distribution of fetal breathing, eye movements, and micturition during active and quiet cycles throughout the last trimester,[5] but there have been no detailed studies of the variations and consistencies in behaviour of healthy or compromised fetuses.

The aim of this study was to document variations in behaviour in a group of healthy mature fetuses.

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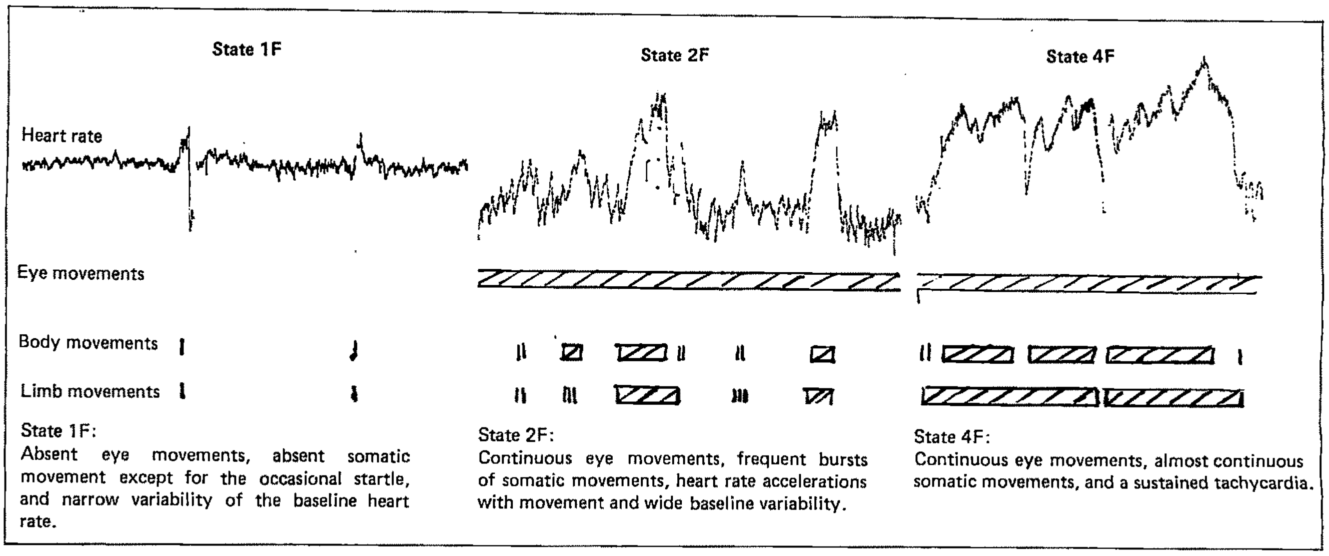
Patients and methods

Forty primigravidas and 42 multigravidas with uncomplicated singleton pregnancies of at least 36 completed weeks' gestation were studied. The observations were conducted in a quiet room between the hours of 10 am and 6 pm. In each case a comfortable semirecumbent position with some lateral tilt was used, and for this purpose we found a Parker Knoll reclining chair was most suitable. The aim was to undertake recordings for at least 100 minutes, because from pilot studies this period of time would allow for at least one cycle of quiet/active behaviour in over 95% of observations (unpublished data). This was not possible in 12 cases (recording time 74–90 minutes) because the mother asked us to stop earlier. All 12 cases had cyclical activity, and there was no evidence that the slightly suboptimal recording times resulted in differences in behavioural findings.

Initial measurements of biparietal diameter, head circumference, and abdominal circumference were noted together with an assessment of liquor volume and fetal normality to confirm that these were low risk pregnancies.

Fetal eye, head, and upper limb movements were detected by one real time ultrasound transducer (Siemens Sonoline SL1, 3·5 MHz transducer or Toshiba SAL 20A, 2·4 MHz transducer) and were stored on video tape, and fetal trunk movements were visualised on a transverse view of the fetal abdomen detected by a second transducer (Toshiba SAL 20A, 2·4 MHz transducer) and recorded verbally on the sound channel of the video recorder. The fetal heart rate was simultaneously recorded with a cardiotocograph (Hewlett Packard 8040A) at a paper speed of 1 cm/minute. All movements and the heart rate were additionally recorded on a polygraph at a paper speed of 3 cm/minute using multiple hand held push buttons, and the



State 1F:
Absent eye movements, absent somatic movement except for the occasional startle, and narrow variability of the baseline heart rate.

State 2F:
Continuous eye movements, frequent bursts of somatic movements, heart rate accelerations with movement and wide baseline variability.

State 4F:
Continuous eye movements, almost continuous somatic movements, and a sustained tachycardia.

Fetal actogram at 39 weeks' gestation.

accuracy of this technique was checked on subsequent playback of the video tape. With this configuration of equipment little interference was obtained using all three transducers simultaneously.

The recordings were used to ascertain the association of the fetal heart rate pattern, eye movements, and somatic movements. The baseline variability of the heart rate pattern was calculated as the mean amplitude of oscillations in the baseline heart rate pattern.

Statistical comparisons between the states, gestational age, time of day, and characteristics of the different heart rate patterns were made by analysis of variance. Ethical approval was obtained from the hospital's research and ethics committee.

Results

Two recordings had to be excluded from analysis of behaviour patterns because visualisation of fetal eye movements was unsatisfactory for 70 to 80% of the recording. The analysis is therefore of the data from 80 fetuses. The total duration of observation sessions was 138·2 hours (mean 103·7 minutes, range 74 to 137 minutes/recording).

BEHAVIOURAL PATTERNS

We identified three different behavioural patterns—two active patterns (2F and 4F), and one quiescent pattern (1F) (figure). Seventy seven of the 80 fetal recordings contained alternating cycles of quiescence and activity. In the remaining three recordings the fetuses sustained a high degree of activity throughout.

Quiescence (state 1F)

A total of 121 episodes of quiescence were recorded (table 1). Three fetuses did not have an episode of quiescence during the recording interval. Seventeen recordings contained an episode of quiescence lasting 30 minutes or more, and the maximum duration recorded was 38 minutes (once in each of two recordings). In 44 recordings more than one episode of quiescence occurred and these have been examined for variability between fetuses.

Characteristics of the accompanying heart rate pattern are shown in table 2. The baseline heart rate and its variability were not always consistent throughout an episode of quiescence or between successive episodes, and the average values for each fetus have been estimated. Ninety three episodes of quiescence were interrupted by startles, and 28 episodes were completely quiescent (the longest episode of complete quiescence being 38 minutes). The mean frequency of startles was one every 8·5 minutes, but within individuals startles seemed to occur at random. Startles were nearly always accompanied by an acceleration in fetal heart rate. There were no eye movements during

Table 1 Number and duration of each behavioural state in 80 fetuses of 36 to 42 weeks' gestation

	Behavioural state				
	1F	*2F*	*4F*	*Indeterminate*	*Total*
No of episodes	121	150	21	25	317
Mean (SD) duration of episode (mins)	20·8 (8·6)	31·6 (19·5)	37·8 (30·4)	9·2 (5·6)	26·1 (18·1)
Range	3–38	3–94	4–137	3–20	3–137
Total (%) time spent in each state (mins)	2516 (30·2)	4785 (57·5)	794 (9·5)	231 (2·8)	8326

State 1F=no eye movements, no somatic movement except for the occasional startle, and narrow variability of the baseline heart rate. State 2F=continuous eye movements, frequent bursts of somatic movements, heart rate accelerations with movement, and wide baseline variability. State 4F=continuous eye movements, almost continuous somatic movements, and a sustained tachycardia.

Table 2 Characteristics of heart rates in each behavioural state

	Behavioural state			
	1F (n=77)	*2F (n=79)*	*p Value**	*4F (n=16)*
Mean (SD) baseline heart rate (beats/min)	133·1 (8·3)	133·5 (7·9)	0·38	160·1 (3·7)
Range	115–150	115–153		155–172
Mean (SD) baseline variability (beats/min)	5·4 (2·0)	11·4 (2·9)	<0·001	Sustained tachycardia not baseline rate so variability not analysed
Range	2–10	4–19		

State 1F=no eye movements, no somatic movement except for the occasional startle, and narrow variability of the baseline heart rate. State 2F=continuous eye movements, frequent bursts of somatic movements, heart rate accelerations with movement, and wide baseline variability. State 4F=continuous eye movements, almost continuous somatic movements, and sustained tachycardia. *Analysis of variance, 1 df.

episodes of quiescence except occasionally during startles. These intervals of quiescence fulfil the criteria for behavioural state 1F described previously.[3,4]

Activity (states 2F and 4F)

A total of 171 active cycles were observed, and accounted for 67% of the total recording time (table 1). The most common behavioural pattern was characterised by frequent bursts of body and limb movements lasting for 20–40 seconds, with intervals of 40–80 seconds between each burst, and occasionally frequent isolated movements. Bursts of movement were nearly always accompanied by an acceleration in fetal heart rate. Between bursts of movement, the baseline heart rate was similar to that of the preceding or succeeding quiescent cycle, but within individuals the baseline variability was significantly wider than during quiescence ($p<0{\cdot}001$). Between individual fetuses there was considerable variation in the baseline rates and variability of the different states, which precluded use of numerical definitions.

There were no significant differences among gestational ages within the range 36 to 42 weeks (table 3).

Three fetuses had baseline heart rates and variability that were similar in activity and quiescence, but in two the different states were still readily distinguishable by the more regular oscillations of the baseline value in quiescence compared with that during activity. In the remaining fetus such episodes could only be distinguished by consideration of eye and somatic movements as well. Sixteen fetuses had one or more intervals of sustained almost continuous activity, with the accompanying heart rate accelerations tending to become fused to a sustained tachycardia, so obscuring the baseline value. These episodes accounted for 9% of the total recording time. In one fetus this high degree of activity persisted for the entire duration of recording so it was extended to determine the maximum length of such intervals, but had to be abandoned after 137 minutes. These two types of active behavioural patterns fulfil the criteria for states 2F and 4F previously described.[3,4]

Indeterminate behaviour

During 3% of the study time fetal behaviour was indeterminate, the variables not conforming to any one of the recognised states for a least three consecutive minutes. About half of these episodes occurred at the time of transition between different states.

Other behavioural patterns

Previous authors have described a fourth behavioural state—'3F'—in which eye movements are present, there are no somatic movements, and the heart rate pattern has a wider baseline variability than in 1F but a more regular oscillation frequency than in 2F.[3,4] We were unable to confirm the existence of this state in the human fetus. Indeed in 138 hours of recording there was only one interval in which eye movements were not associated with somatic movements for three minutes or more. This epoch lasted for four minutes. It was observed during a sustained period of 2F, and the accompanying fetal heart rate pattern had a baseline oscillation frequency that was characteristic of—and indistinguishable from—that of the preceding and succeeding 2F fetal heart rate pattern.

State transitions

It has been suggested that before a fetus can be

Table 3 Trends in incidence of behavioural states and heart rates by gestational age from 36 to 42 weeks

	Gestational age (weeks)			
	36–37 (n=15)	*37–38 (n=24)*	*38–39 (n=19)*	*>39 (n=22)*
Mean (SD) percentage in state 1F	28·6 (9·5)	29·7 (15·0)	31·2 (11·7)	33·3 (13·7)
Mean (SD) percentage in state 2F	60·5 (12·3)	55·8 (16·7)	60·1 (17·7)	52·5 (23·8)
Mean (SD) percentage in state 4F	7·0 (15·0)	11·4 (20·3)	6·2 (16·9)	10·2 (26·6)
Mean (SD) percentage in indeterminate state	2·3 (5·8)	3·2 (5·3)	4·8 (5·5)	1·1 (2·7)
Mean (SD) basal heart rate in state 1F	132·4 (9·4)	133·4 (8·2)	133·0 (8·4)	133·5 (7·9)
Mean (SD) basal heart rate in state 2F	131·5 (7·0)	133·5 (8·7)	134·5 (7·9)	134·3 (7·8)
Mean (SD) baseline variability in state 1F	6·7 (2·2)	4·7 (2·1)	5·2 (1·5)	5·3 (2·0)
Mean (SD) baseline variability in state 2F	11·1 (3·1)	10·8 (3·0)	12·6 (2·2)	11·3 (3·0)

State 1F=no eye movements, no somatic movement except for the occasional startle, and narrow variability of the baseline heart rate. State 2F=continuous eye movements, frequent bursts of somatic movements, heart rate accelerations with movement, and wide baseline variability. State 4F=continuous eye movements, almost continuous somatic movements, and sustained tachycardia. There are no significant differences among the groups.

Table 4 Influence of recording time on behaviour

	Behavioural state			
	1F	*2F*	*4F*	*Indeterminate*
Mean (SD) percentage time spent in each state by each fetus				
Morning (n=35)	32·4 (13·0)	57·7 (16·9)	9·1 (17·7)	2·8 (4·5)
Afternoon (n=45)	29·8 (12·9)	57·2 (18·7)	8·1 (21·7)	2·7 (5·3)

State 1F=no eye movements, no somatic movement except for the occasional startle, and narrow variability of the baseline heart rate. State 2F=continuous eye movements, frequent bursts of somatic movements, heart rate accelerations with movement, and wide baseline variability. State 4F=continuous eye movements, almost continuous somatic movements, and sustained tachycardia. There are no significant differences among the groups.

considered to have behavioural states, the transitions from one state to the next should not take longer than three minutes.[3 4] We felt that is was only valid to consider transitions into and out of 1F (quiescence), as only these transitions require a change in the condition of all three variables. We observed 181 such transitions (11 transitions in which there was poor visibility of the fetal eye have not been included) and found that these transition times varied between 5 and 1320 seconds. We noted a trend towards shorter transition times with advancing gestation within the ages studied, but these differences were not significant (p=0·572).

Individual fetuses showed considerable variation in transition times between states (5 to 520 seconds). In view of this variation we considered that to implement a 'three minute transition time' rule for distinguishing fetuses with and without behavioural states was arbitrary, unnecessarily complicated, and confusing. Ignoring the 3 minute transition rule, we found a high percentage of linkage between the variables as behavioural states, and a low incidence of indeterminate behaviour (table 1).

EYE MOVEMENTS

We found that fetal eye movements could be recognised easily with a little practice, but unsatisfactory visualisation of the fetal eye was the main limitation in the recording and analysis of fetal behavioural states, resulting in our being unable to study two of the 82 fetuses at full term. This problem usually occurred when the fetal head was in the direct occipitoanterior position, when it was quite impossible to visualise the eye even with the head unengaged. Conversely when the head was in the occipitotransverse or occipitoposterior positions, good visualisation could be obtained even when the head was deeply engaged.

TIME OF DAY

We did not attempt to examine diurnal variations in behavioural states, but over the range of times included, recordings beginning before and after noon fell into two groups of similar size, and these were compared (table 4). The only apparent trend was for more quiescence (1F) in morning than afternoon studies, but this was not significant.

NEONATAL OUTCOME

All birth weights were between the 10th and 90th percentile for sex and gestational age. All infants had Apgar scores at one minute above 5, and Apgar scores at five minutes above 8. None required admission to the neonatal intensive care unit, and the only neonatal problem before discharge was moderate jaundice in two infants, which was treated with phototherapy.

Discussion

The potential importance of accurate documentation of behavioural patterns and their development in the human fetus lies in enhancement of our understanding of the aetiology and pathophysiology of neurodevelopmental handicap that develops before birth.

The main clinical implication of these studies is that they emphasise the need to redefine normal limits for the biophysical tests of fetal well being currently in use. For example, conventional biophysical profile scoring requires three fetal movements and two fetal heart rate accelerations in a 30 minute period.[6] Twelve of the 80 recordings in this study contained episodes of 1F lasting 30 minutes or longer without two or more startles, and these healthy fetuses would not have achieved a normal score had testing occurred during these intervals. In contrast, three movements should occur in minutes during an interval of 2F, and in seconds during an interval of 4F. The same limitations apply to the non-stress test.

Recently a number of papers about auditory stimulation of the fetus have been published, but only one group of authors have looked at results in relation to the fetal behavioural state; they found that the results were dependent on behaviour state.[7] The indices of Doppler recording of fetal blood flow also seem to be dependent on behaviour state.[8–10] The importance of recognising this dependence is well established in neurological assessment of the newborn and should not be ignored in any tests involving stimulation of the fetus.[11–13]

We have confirmed the existence of three of the four behavioural states previously described in a group of 80 healthy fetuses of 36 or more completed weeks' gestation. We were unable, however, to find a state 3F (eye movements present, no body movements, and a fetal heart rate pattern with a wider oscillation frequency than in 1F but with more regular oscillations than in 2F) in any of the recordings. To explain this we considered whether the use of a paper speed of 1cm/minute (the standard recording speed used in most units in the United Kingdom) rather than 3cm/minute (as used in The Netherlands) might account for our failure to differentiate this heart rate pattern. As patterns accompanying states 1F, 2F, and 4F could clearly be differentiated, however, and were exactly as described in recordings performed at 3 cm/minute, we felt that this pattern should have been distinguishable. Furthermore, we have carried out some recordings subsequently at a paper speed of 3 cm/minute and found that the differences between patterns accompanying the different states are if anything less apparent. We still cannot recognise this pattern.

The more likely explanation for our failure to find state 3F is that it occurs infrequently, if at all, in the fetus. We note that in previous studies it was found in only a minority of fetuses, and for short intervals, typically of three to six minutes duration.[3 4]

The main practical problem we encountered was our inability to observe fetal eye movements when the fetal head was in the direct occipitoanterior position, and we could not find any means of overcoming this except by waiting for the fetus to change position.

Consideration of ultrasonically recorded measurements as either linked behavioural patterns ('states') or indeterminate behaviour, rather than 'states', 'coincidence' and 'no-coincidence' as described by previous authors,[3 4] simplifies the analysis and allows interpretation of shorter recordings, which might be of greater clinical relevance. It is also likely to make interpretation of recordings at earlier gestational ages easier, when linkage between the variables is less consistent (unpublished observations).

Clearly these behavioural cycles—of what seem similar to quiet sleep and active states in the newborn—are well developed in the normal human fetus at full term, and may indicate integrity and maturity of neurological development quite unlike any other biophysical investigations. For the time being the clinical applicability of such recordings is limited, firstly because both the recordings and their analysis are extremely time consuming, and secondly availability of all the ultrasound equipment required is likely to be limited.

As yet, however, there is little information on the sequence of normal development of behaviour, the influence of antenatal complications, and the association with long term neurodevelopmental outcome. For these reasons such behavioural studies remain of central importance in the research of perinatal neurological development.

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Access for peritoneal dialysis in neonates and infants

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Abstract

A new catheter for peritoneal dialysis in neonates and infants was used on 28 occasions in 17 patients. Advantages over other catheters included easy safe introduction over a guide wire, absence of early leakage of dialysate, and the ability to change the catheter without creating a second abdominal wound.

Acute renal failure is a common complication of severe illness in neonates and infants and the treatment of choice is early peritoneal dialysis.[1] One difficulty is the introduction of a suitable peritoneal catheter. Several catheters and techniques have previously been described in neonates,[2–4] all of which require the introduction of a plastic catheter into the peritoneum, either over a trocar (as in adult acute peritoneal dialysis) or over a stylet (as when inserting an intravenous cannula). Acute peritoneal dialysis using a stiff catheter introduced into the peritoneum over a guidewire has been described in adults.[5] We describe a new catheter specifically for use in neonates and infants, which uses this technique of introduction and which we feel has several advantages over other catheters.

Methods

CATHETER

The 'Pendlebury' catheter (Medcomp) is based on a percutaneous haemodialysis catheter. It is made of polyurethane, 14 gauge internal diameter, and 5·1 or 8·9 cm long. There are four side holes, the most proximal being 2·6 cm from the distal end. The catheter itself is moulded to a short soft plastic extension tube that terminates in a female Luer lock connection. It is introduced by the Seldinger technique over a 'j' wire, as described below. Free gravitational flow rates of 140 ml/min at a height of 100 cm can be achieved, allowing efficient dialysis in children weighing up to 14 kg with standard volumes.

INSERTION

After choosing an appropriate site to introduce the catheter, the skin is prepared with a solution containing iodine or chlorhexidine. The best site for introduction will vary from child to child depending on the presence of operation scars or enlargement of organs, or both. On the whole we prefer to introduce the catheter at the level of the umbilicus just lateral to the rectus sheath, but it can be introduced anywhere along a line parallel to the rectus sheath, or in the midline below the umbilicus.

After preparation of the skin, local anaesthetic (1% lignocaine) is injected into the site, and a 22 gauge IV cannula introduced. This is primed with saline and then removed.

The pink hubbed introducer needle is then inserted straight through into the peritoneum. This is flushed with 5 ml normal saline and aspirated to ensure bowel content is not retrieved (fig 1). This needle is left resting in the peritoneum, and the 'j' tip of the guidewire is straightened using the blue plastic adapter on it (fig 2). This adapter is then placed into the hub of the introducer needle and the wire advanced through the needle into the peritoneum (fig 3).

As the 'j' end of the wire emerges through the tip of the needle a 'give' is felt as the resistance against the advancing wire is reduced. The wire should now advance a further 1–4 cm without any resistance. If there is resistance, the tip of the wire might either be in bowel or in subcutaneous tissue, in which case the needle and wire can be removed and the procedure started again. With the wire resting in the peritoneum the blue plastic adapter and needle are removed.

The catheter is now threaded over the wire (fig 4), in preterm infants there is no need to make a skin incision—the catheter can simply be forced through with a 'screwing' action. There is no risk of the catheter causing intra-abdominal injury as it will simply follow the wire. In the infant born at full term, and in older infants, a 3–4 mm skin incision around the wire will make passage through the skin easier.

Once the catheter has been inserted the wire is removed, the catheter is connected to a peritoneal dialysis delivery system, and the soft plastic extension of the catheter is taped to the skin.

Results

Dialysis was carried out in 17 patients ranging from 27 weeks' gestation to 2·7 years of age and from 800 g to 13 kg in weight (tables 1 and 2). Ten of the 17 patients were neonates, of whom four had severe respiratory distress complicated by hypoxia and sepsis, three had mainly surgical problems, two had inborn errors of metabolism, and one had aortic thrombosis complicating umbilical arterial catheterisation. Haemolytic uraemic syndrome was the most common cause of renal failure outside of the neonatal period.

The mean duration of dialysis was 6·2 days (range 0·21 to 34). Seven of the 17 patients (41%) required more than one catheter. There were 22 problem incidents giving an overall

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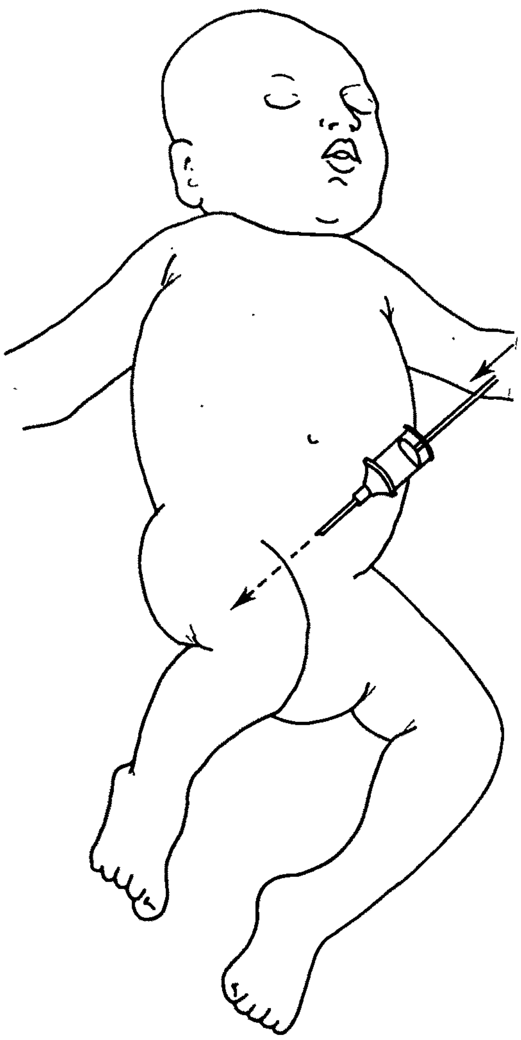

Figure 1 Insertion of the introducer needle into the peritoneum.

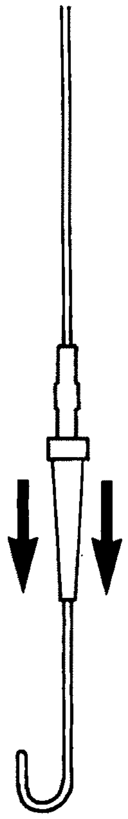

Figure 2 Straightening 'J' wire with blue plastic adaptor.

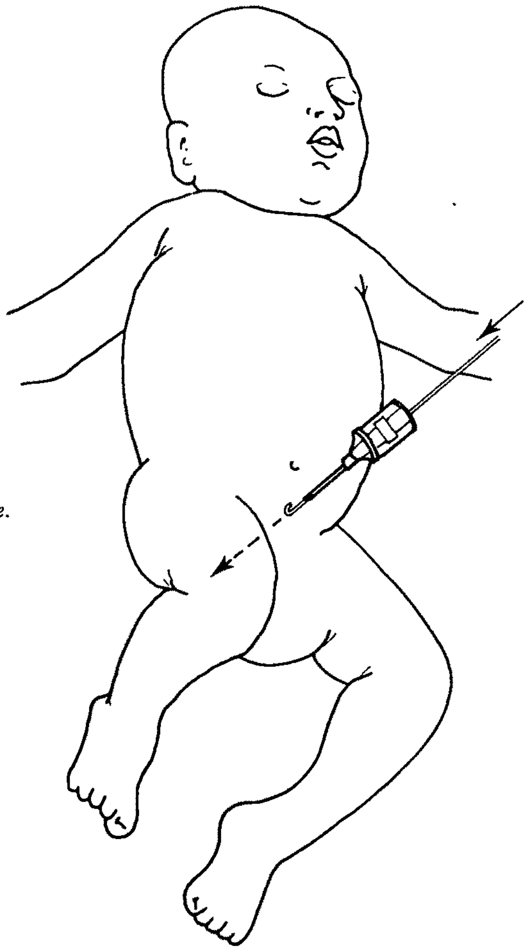

Figure 3 Passage of wire into peritoneum through needle.

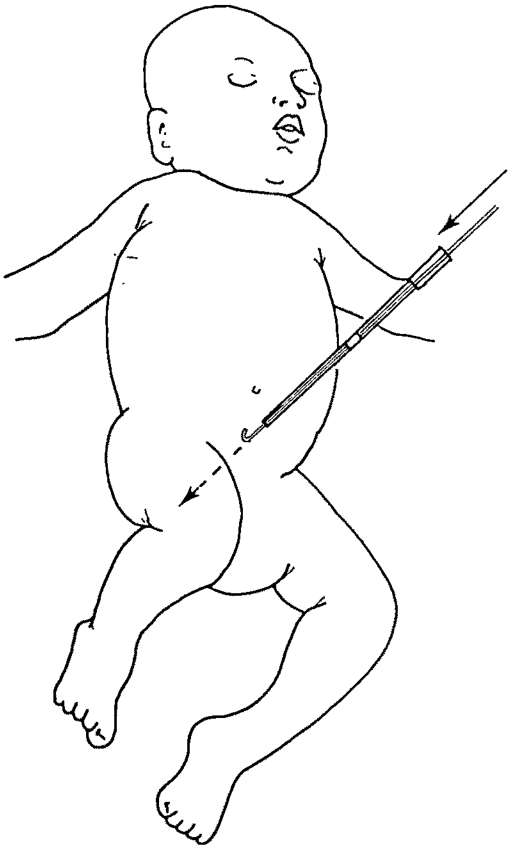

Figure 4 Passage of catheter over wire into peritoneum.

Table 1 Details of neonates studied

Case No	*Gestation (weeks)*	*Age (days)*	*Weight (g)*	*Diagnoses*	*Duration of peritoneal dialysis (days)*	*Outcome*
1	40	27	3500	Aortic thrombosis	34·0	Survived, now in end stage renal failure
2	38	28	2200	Hepatorenal failure, undiagnosed cause	11·0	Died
3	39	1	2800	Primary lactic acidosis	5·2	Died
4	26	2	800	Asphyxia, severe idiopathic respiratory distress syndrome	0·21	Underwent diuresis died six days later
5	27	11	1100	Septicaemia, disseminated intravascular coagulation	0·33	Died
6	37	27	1600	Gastroschisis, disseminated intravascular coagulation	0·5	Died
7	28	11	1100	Necrotising enterocolitis, septicaemia	6·0	Died
8	27	2	1000	Asphyxia, severe idiopathic respiratory distress syndrome	1·0	Died
9	30	1	1500	Idiopathic respiratory distress syndrome, hypotension	3·0	Alive and well
10	32	9	1800	Exomphalos, septicaemia	9·0	Died

Table 2 Details of older infants studied

Case No	*Age (months)*	*Weight (kg)*	*Diagnoses*	*Duration of peritoneal dialysis (days)*	*Outcome*
11	32	13·0	Haemolytic uraemic syndrome, septicaemia	3·0	Died
12	15	10·0	Haemolytic uraemic syndrome	11·8	Alive and well
13	3	5·1	Haemolytic uraemic syndrome	5·0	Alive and well
14	1	4·2	Hypernatraemic dehydration	0·6	Alive and well
15	10	7·9	Haemolytic uraemic syndrome	10·8	Alive and well
16	6	4·4	Septicaemia, disseminated intravascular coagulation	2·0	Died
17	1·7	5·0	Multisystem failure—? 'cot death'	1·9	Died

complication rate of 0·21 episodes/patient day. Blockage or poor drainage occurred with 12 catheters but in only six was either the reason for removal. There was no early leakage of dialysate (within 48 hours of insertion). Some late leakage occurred with seven catheters, although it was an appreciable amount in only four cases. There were three episodes of peritonitis, two of which occurred in one patient. Twenty eight catheters were used of which 12 (43%) were removed because of problems: six because of blockage, four because of late leakage, and two because of peritonitis. The mean duration of function of these catheters was 4·1 days (range 0·5 to 11). For the 16 catheters removed electively the mean duration of function was three days (range 0·21 to 9).

One infant with repaired gastroschisis who weighed 1060 g developed peritonitis, septicaemia, and an ileus, after a catheter had eroded into the wall of diseased bowel. This was discovered at necropsy; the catheter had worked well until a few hours before death when treatment was electively stopped. There were no other cases of injury, even in infants who had had abdominal operations.

Discussion

The 'Pendlebury' catheter has several advantages. It is soft and pliable (particularly at body temperature), but it does not kink. Using a 'j' wire makes introduction safer because the wire will not advance easily if the tip of the introducing needle is in subcutaneous tissue or in a viscus rather than in the peritoneal cavity. This means that priming of the abdomen is not essential and can be omitted if it is felt the patient would not tolerate high intra-abdominal pressures. The tissues are stretched around the catheter so that there is a tight fit and no early leakage. After it has been sited the catheter is taped flat to the skin, so making nursing easier, particularly in mobile infants. The catheter is small enough for one to be inserted on each side of the abdomen and continuous cross flow dialysis carried out.

Blockage or poor drainage can be managed either by flushing with saline, or, if this is unsuccessful, by passing a guidewire through the catheter until it is beyond the tip, removing the catheter and inserting a new catheter over the guidewire. This almost invariably establishes good flow without the problem of leakage from an old catheter site.

There are few data available about the incidence of complications in this age group with other peritoneal dialysis catheters. We have found that thin walled intravenous cannulas block and kink easily. The same applies to the 'Wallace' peritoneal dialysis catheter,[2] which has two additional problems. Being introduced over a sharp stylet gives almost no 'feel' of where one is in the abdomen, and having side holes extending back 5 cm from the tip makes full introduction difficult in a small abdomen. Small neonatal chest drains are just as difficult to introduce into infant abdomens as standard trocar catheters.

Compared with catheters introduced over trocars, the incidence of problems in our group was similar to that found by Wong and Geary in their group of 33 patients (mean age 3·4 years, mean weight 15·1 kg) treated with Trocath catheters,[6] despite the fact that our patients were appreciably smaller and younger (table 3). Obstruction to flow seems to be more common with the Pendelbury catheter, but is more easily dealt with. Eight out of 12 patients in the series described by Wong and Geary required removal of their catheters because of obstruction to flow, compared with four out of 10 in our series.

Table 3 Comparison of the results obtained using the Pendlebury and the Trocath catheter[6]

	Trocath catheter (n=33)	*Pendlebury catheter (n=17)*
Mean duration of dialysis (days)	7·1	6·2
Overall complication rate (episodes/patient/day)	0·19	0·21
No (%) patients having catheter removed because of problems	19 (58)	8 (47)
No (%) patients requiring two or more catheters	12 (36)	7 (41)
No (%) with leakage of dialysate	12 (36)	6 (35)
No (%) with obstruction to flow	12 (36)	10 (59)
No (%) with peritonitis	5 (15)	2 (12)

Leakage of dialysate was equally as common in both groups, but did not occur with the Pendlebury catheter until after four days of dialysis, whereas with the Trocath catheters early leakage occurred in four patients. Early leakage is a much more difficult problem than late leakage, as it can prevent successful dialysis at an early stage when the patient's biochemical state is at its worst and dialysis is most required.

We feel that the Pendlebury catheter is an important improvement on other catheters that are available for peritoneal dialysis in neonates and children up to 18 months old, judged to a large extent on clinical impression. The time has now come to carry out a controlled trial of different catheters and means of peritoneal dialysis in small ill infants.

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Immunoglobulin and anti-Escherichia coli antibody in lower respiratory tract secretions from infants weighing less than 1500 g at birth

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Abstract

Concentrations of immunoglobulins and anti-*Escherichia coli* antibody were studied longitudinally in tracheobronchial aspirates from 33 premature intubated neonates, median gestational age 27 weeks. Aspirates collected at birth contained IgG, IgA, and IgM in 100%, 93%, and 79% of samples, respectively. The median IgA concentration at birth was 0·7 μg/mg total protein and increased to 5·8 μg/mg protein by the sixth week. IgG and IgM antibodies to *E coli* were present in 90% and 30%, respectively, of tracheobronchial aspirates collected at birth. Samples from three of 28 neonates (11%) contained IgA anti-*E coli* antibody at birth, and the proportion with IgA antibody rose to 50% during the sixth week. Secretory component associated IgA and IgM were detectable in samples tested at birth and at 4 weeks of age, and secretory component associated anti-*E coli* antibody was present in aspirates from three of nine neonates studied at 4 weeks of age, but had not been detectable at birth.

Premature neonates often require respiratory support, and during this time the respiratory tract rapidly becomes colonised.[1] Bacterial colonisation of the respiratory tract is associated with an increased incidence of systemic infection,[2] which contributes to the high morbidity and mortality of very low birthweight infants.[3 4] Antibodies in secretions play an important part in protecting the infant from infection by preventing adherence of antigen and by neutralising organisms or their products.[5] Although the immune system of the airways and lungs in adults and older children has been well studied,[6–9] little is known of the respiratory defence mechanisms in neonates and infants.[10 11] Forsyth *et al* have shown in premature neonates that concentrations of IgA and IgM in tracheobronchial lavage increase with increasing postnatal age.[11] Intubated premature neonates have also been shown to produce IgM antibody in serum to bacteria present in the lower respiratory tract.[1] The presence of specific antibody in lower respiratory tract secretions, however, and the association of the appearance of specific antibody in secretions and postnatal age in very premature neonates are unknown.

In this prospective study we investigated the origin and development of immunoglobulin and specific antibodies in tracheobronchial aspirates from very premature neonates. Total IgG, IgA, and IgM concentrations and anti-*Escherichia coli* IgG, IgA, and IgM antibody concentrations in tracheobronchial aspirates were determined longitudinally up to 5·5 weeks after birth in a cohort of 33 intubated premature neonates whose gestational ages ranged from 25 to 30 weeks.

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Patients and methods

Thirty three premature infants (13 boys, 20 girls) who required immediate postnatal intubation and ventilation were enrolled in a prospective study. The birth weights ranged from 674 to 1500 g (median 1001) and the median gestational age assessed by serial ultrasonography and clinical examination was 27·0 weeks (range 25–30). The indications for artificial ventilation in all 33 neonates were prematurity and hyaline membrane disease. The duration of intubation ranged from 14 to 39 days (median 27).

The position of the endotracheal tube was determined by chest radiographs, the tip of the tube being positioned 1–2 cm above the tracheal carina. Tracheobronchial aspirates were collected at the time of intubation and at weekly intervals (within one day) thereafter. Samples were collected through the endotracheal tube by gentle suction after instillation of up to 0·2 ml of sterile normal saline followed by ventilation for 15 to 30 seconds. The mean volume of aspirate collected was 200 μl (range 50–400 μl/sample). Aspirates containing blood were discarded. Samples were stored in polypropylene containers at −70°C until analysed. Cord blood was collected from 23 of the 33 preterm infants at delivery. The serum samples were also stored in polypropylene containers at −70°C until analysed.

PREPARATION OF ASPIRATES

Before analysis, all aspirates were thawed and sonicated (Branson Sonifier 450) at 100% output for at least one minute. The samples were then centrifuged at 1500 *g* for 10 minutes, and the supernatant collected and used in the assays described below.

QUANTITATION OF TOTAL CONCENTRATIONS OF IGG, IGA, AND IGM

Enzyme immunoassays for the measurement of total concentrations of IgG, IgA, and IgM in serum samples from cord blood were carried out as previously described.[12] All samples were tested in duplicate at four dilutions. A pooled human serum reference preparation containing 8·064 g IgG/l, 1·449 g IgA/l, and 0·966 g IgM/l was tested at tenfold dilutions ranging from $1/10^3$ to $1/10^8$ on each plate.[13] With this assay IgG was detectable to 2×10^{-7} g/l, IgA to 4×10^{-7} g/l, and IgM to 3×10^{-7} g/l.

Enzyme immunoassays for the estimation of total concentrations of IgG, IgA, and IgM in tracheobronchial secretions were carried out as previously described.[12] All samples were tested in duplicate at three dilutions. A reference preparation of pooled multiple tracheobronchial aspirates from two patients aged 6 years and 8 years (who had permanent tracheostomies for management of high spinal injuries) was included on each assay plate in fivefold dilutions ranging from 1/500 to 1/1562500 so that a standard curve for the determination of relative units for each test sample could be derived. The concentrations of IgG, IgA, and IgM/mg total protein in this reference preparation were 58·2 μg, 159·1 μg, and 20·9 μg, respectively. The lower limit of sensitivity was $3{\cdot}9\times10^{-7}$g/l, $3{\cdot}6\times10^{-7}$g/l, and $1{\cdot}4\times10^{-7}$g/l for IgG, IgA, and IgM, respectively.

Enzyme immunoassays for the determination of secretory component associated immunoglobin were carried out by modifications of the above assays in which a murine monoclonal antisecretory component antibody (Australian Monoclonal Developments, Sydney) was used to detect secretory component associated IgM or IgA bound to the solid phase by affinity isolated anti-IgM or anti-IgA precoating antibody (Tago, Burlingame, California). Affinity isolated peroxidase conjugated antimouse IgG was used to develop the substrate reaction.

ANTIBODY TO POOLED *E COLI* ANTIGENS

Enzyme immunoassays were used to measure concentrations of IgG, IgA, and IgM antibodies specific to *E coli* polysaccharide in all samples of tracheobronchial fluid and cord serum. A pool of eight strains of *E coli* representing strains found most frequently in faecal isolates or diseases (serotypes 01, 02, 04, 06, 07, 016, 018, and 075) was prepared by a heat extraction technique.[14 15]

Ninety six well microtitre plates (Nunc) were incubated with 100 μl of *E coli* antigen in 0·05 M bicarbonate buffer (pH 9·5; final concentration 0·09 μg *E coli* antigen/ml) in each test well for two hours at 37°C and then overnight at 4°C. Residual binding sites were blocked with 50 μ/well 2·5% skim milk powder in 0·05 M bicarbonate buffer (pH 9·5) for two hours at 37°C.[16] After washing, 50 μl/well of sample diluted in phosphate buffered saline containing 0·05% Tween 20 (PBS-T) (pH 7·2) and containing 2·5% skimmed milk powder (SMP) was added and incubated for two hours at 37°C. After washing, 50 μl of a 1:1000 dilution of peroxidase conjugated, affinity isolated, goat antihuman IgG, or IgA, or IgM (Tago), in PBS-T/SMP was added and incubated for two hours at 37°C. The plates were then washed and 50 μl of orthophenylene diamine substrate was added. The reaction was stopped after incubation for 15 minutes in the dark by adding 25 μl of 8N hydrochloric acid solution to each well. Absorbance values were read at 492 nm using a Titertek Multiscan (Flow Laboratories).

All samples were tested in duplicate and at three dilutions ranging from 1/10 to 1/250 for tracheobronchial fluid samples, and from 1/10 to 1/1250 for cord samples. The pooled human reference serum[13] and the tracheobronchial fluid reference preparation were each included in six serial dilutions on every test plate. Each test sample was quantitated by reading from interpolated points generated from the linear portion of the appropriate reference standard curve. The concentration of anti-*E coli* antibodies in the reference preparations was estimated using a modification of a method previously described.[17] The pooled reference serum contained $0{\cdot}42\times10^{-3}$g IgA anti-*E coli* antibody/l, $3{\cdot}43\times10^{-3}$g IgG antibody/l, and $6{\cdot}98\times10^{-3}$g IgM antibody/l, whereas the tracheobronchial fluid reference preparation contained 31·01 ng IgA anti-*E coli* antibody/mg total protein, 6·25 ng IgG antibody/mg total protein, and 51·14 ng IgM antibody/mg total protein.

Specificity of the assays for IgG, IgA, and IgM antibodies to the *E coli* antigens was illustrated by appropriate liquid phase absorption experiments using the reference sample preparations and the pooled *E coli* antigen preparation. The lower limit of detection was taken at optical density values of more than twice the background (sample free) optical density readings. The lower limit of IgG, IgA, and IgM anti-*E coli* antibody detection (g/l) for the serum reference preparation was $1{\cdot}2\times10^{-7}$, $1{\cdot}0\times10^{-7}$, and $0{\cdot}9\times10^{-7}$, respectively, and $0{\cdot}3\times10^{-7}$, $0{\cdot}1\times10^{-7}$, and $0{\cdot}1\times10^{-7}$ for the tracheobronchial fluid reference preparation.

Secretory component associated antibody to the pooled *E coli* antigens was determined by appropriate modification of a previously described assay.[12]

TOTAL PROTEIN QUANTITATION

Measurements of total protein concentration were made by the method of Lowry *et al.*[18] To correct for any errors introduced by sample dilution during endotracheal tube aspiration, the concentration of tracheobronchial immunoglobulin or specific antibody in samples was expressed as a ratio of μg of immunoglobulin or ng of antibody/mg of total protein in the sample.

STATISTICAL ANALYSIS AND ETHICS

Results in the tables are given as medians for all samples. As age increased after birth fewer neonates remained intubated. Statistical comparisons of results at differing postnatal ages were made using paired samples only. Analyses of immunoglobulin concentrations in paired samples were done by the Wilcoxon matched pairs signed rank test and the Spearman rank order correlation analysis. Longitudinal comparisons of proportions of study samples with detectable anti-*E coli* antibody were done with the McNemar test for the significance of changes.

The study was approved by the institutional human ethics committees for each of the three participating neonatal intensive care units. Informed written consent was obtained from the parents of all neonates before enrolment in the study.

Results

TOTAL IGG, IGA, AND IGM CONCENTRATIONS

All samples of cord serum contained IgG, IgA, and IgM. The median concentrations for IgG, IgA, and IgM were 4·87 g/l (range 1·82–9·52 g/l), 0·004 g/l (range 0·001–0·04 g/l), and 0·07 g/l (range 0·04–0·58 g/l).

A total of 144 tracheobronchial aspirates from 33 premature neonates were collected and analysed. The median immunoglobulin concentrations/mg total protein in tracheobronchial fluid samples at each postnatal collection time are shown in table 1. All aspirates contained IgG, with the highest concentrations present at birth (table 1). Two weeks later, the median IgG concentration had decreased significantly (Wilcoxon matched pairs signed rank test, 24 pairs, $p<0{\cdot}03$), and the median IgG concentration at 5·5 weeks remained significantly lower than at the time of perinatal intubation (10 pairs, $p<0{\cdot}02$).

The median IgA concentrations in the tracheobronchial aspirates are shown in table 1. IgA was detectable in 26 of the 28 aspirates collected at birth (93%), although the median IgA concentration was low (0·7 μg/mg total protein). The median IgA concentration remained low during the first three weeks, and then showed a progressive increase (Wilcoxon: between three and four weeks after birth, 16 pairs, $p<0{\cdot}04$; between 3 and 5·5 weeks after birth, nine pairs, $p<0{\cdot}03$). The median concentration of IgA in tracheobronchial aspirates at 5·5 weeks of age was 5·8 μg/mg total protein (range 0·3–13·0 μg/mg total protein, n=10).

IgM was detectable in samples from 22 of the 28 neonates studied at birth (79%). Within the first week the median IgM concentration increased significantly (Wilcoxon; 24 pairs, $p<0{\cdot}001$), but there was no further significant change thereafter.

The ratio of the median concentration of total IgA: total IgM in tracheobronchial aspirates increased significantly after the second week (Wilcoxon: week 2 to week 3, 17 pairs, $p<0{\cdot}01$), reaching a value of 1·0 at about five weeks (Wilcoxon: week 2 to 5·5, eight pairs, $p<0{\cdot}02$).

Using the Spearman rank order correlation analysis, there were no significant correlations between IgG, IgA, and IgM concentrations in samples of cord serum (mg/100 ml) and in tracheobronchial fluids (μg/mg total protein) collected at the time of perinatal intubation (n=23, each $r_s<0{\cdot}38$, each $p>0{\cdot}05$). Similarly, there were no significant correlations between concentrations of tracheobronchial IgG, IgA, and IgM (μg/mg total protein) at delivery and the gestational age at birth (n=28, each $r_s<0{\cdot}21$, each $p>0{\cdot}14$).

ANTIBODY TO POOLED *E COLI* ANTIGENS

The median concentrations of IgG, IgA, and IgM antibodies to the pooled *E coli* antigens in cord samples were $0{\cdot}91\times10^{-3}$g/l (range 0·10–5·08), 0×10^{-3}g/l (range 0–0·01), and $0{\cdot}03\times10^{-3}$g/l (range 0–0·46) respectively.

The median concentrations of anti-*E coli* antibody in tracheobronchial fluid at differing postnatal ages are shown in table 2. Table 3 shows the percentage of analysed tracheobronchial fluid samples with detectable anti-*E coli* antibody within the three isotypes IgG, IgA, and IgM at differing postnatal ages.

Almost all samples contained IgG anti-*E coli* antibody. At the time of intubation, IgG anti-*E coli* antibody was detected in 25 of 28 samples (89%) and the median concentration was 19·1

Table 1 Median (range) concentrations of IgG, IgA, and IgM in samples of tracheobronchial fluid (μg/mg total protein)

	Birth (n=28)	*Week 1 (n=27)*	*Week 2 (n=29)*	*Week 3 (n=21)*	*Week 4 (n=18)*	*Week 5 (n=12)*	*Week 5·5 (n=10)*
IgG	61·5 (25·1–381·5)	57·8 (13·3–238·0)	46·1 (16·4–156·9)	37·1 (12·2–90·5)	27·1 (11·8–81·5)	22·9 (13·4–47·5)	22·6 (5·6–33·5)
IgA	0·7 (0·0–3·5)	0·9 (0·0–6·94)	0·6 (0·0–5·8)	0·7 (0·2–8·4)	1·4 (0·1–10·5)	3·4 (0·3–18·1)	5·8 (0·3–13·0)
IgM	0·6 (0·0–4·3)	2·1 (0·2–21·6)	2·1 (0·2–13·1)	2·6 (0·3–38·8)	2·8 (0·3–8·2)	3·3 (0·2–15·0)	2·9 (0·2–8·5)

Table 2 Median (range) concentrations of IgG, IgA, and IgM anti-E coli antibody in samples of tracheobronchial fluid (ng/mg total protein)

	Birth (n=28)	*Week 1 (n=27)*	*Week 2 (n=29)*	*Week 3 (n=21)*	*Week 4 (n=18)*	*Week 5 (n=12)*	*Week 5·5 (n=10)*
IgG	19·1 (0–189·5)	8·7 (0–218·7)	6·2 (0–72·9)	5·6 (0–34·2)	3·8 (0–22·5)	2·9 (0–16·8)	3·2 (0–16·4)
IgA	0·0 (0–1·6)	0 (0–1·2)	0 (0–10·5)	0 (0–0·6)	0 (0–0·5)	0 (0–19·8)	0·1 (0–54·6)
IgM	0 (0–7·7)	0 (0–5·7)	0·3 (0–8·9)	0·3 (0–1·6)	0·3 (0–1·0)	0·2 (0–2·1)	0·2 (0–2·6)

Table 3 Percentage of tracheobronchial aspirates with detectable amounts of IgG, IgA, and IgM anti-E coli antibody at increasing postnatal ages

	Birth (n=28)	*Week 1 (n=27)*	*Week 2 (n=29)*	*Week 3 (n=21)*	*Week 4 (n=18)*	*Week 5 (n=12)*	*Week 5·5 (n=10)*
IgG	90·2	81·4	83·0	76·4	89·1	75·2	70·3
IgA	11·1	18·5	21·4	23·8	33·3	41·7	50·0
IgM	30·4	40·7	62·1	66·7	61·1	58·3	60·0

ng/mg total protein (range 0–189·5). There was an insignificant decrease to a median value of 3·2 ng/mg total protein (range 0–16·4) at 5·5 weeks (McNemar test for significance of changes, nine pairs, $p>0·1$).

Concentrations of IgA anti-*E coli* antibody ranged between 0 and 19·8 ng/mg total protein during the first five weeks, with median values of 0 ng/mg total protein at each time of sampling up to and including 5 weeks of age. The median value at 5·5 weeks was 0·13 ng/mg total protein (range 0–54·6 ng/mg total protein). The percentage of samples with detectable IgA anti-*E coli* antibody increased as postnatal age increased. IgA antibody was present in 11·1% of samples at birth, and in 50·0% of samples at 5·5 weeks after delivery (McNemar: eight pairs, $p<0·04$).

IgM anti-*E coli* antibody was detectable in 30% of samples at the time of intubation. At two weeks postpartum significantly more neonates (62%) had detectable IgM anti-*E coli* antibody than at birth (McNemar: 28 pairs, $p<0·03$). The median IgM antibody concentration at two weeks was 0·3 ng/mg total protein (range 0·0–8·9 ng/mg total protein); it remained at this value at 3 and 4 weeks after birth, and then fell to a median concentration of 0·17 ng IgM antibody/mg total protein ($n=10$) at 5·5 weeks.

There was a significant correlation between the concentration of IgG anti-*E coli* antibody in paired cord samples and tracheobronchial aspirates at the time of immediate postnatal intubation ($n=21$, $r_s=0·43$, $p=0·02$) but no similar correlation was found for IgA and IgM antibodies. There was no significant correlation between gestational age and concentrations of IgG, IgA, or IgM anti-*E coli* antibodies in tracheobronchial fluids at the time of delivery ($n=28$, all $r_s<0·18$, $p>0·17$).

Concentrations of IgA and IgM anti-*E coli* antibodies did not correlate with total concentrations of IgA or IgM in tracheobronchial aspirates at any of the sampling times. Concentrations of IgG anti-*E coli* antibody in tracheobronchial aspirates correlated significantly with total concentrations of IgG in tracheobronchial fluid during the first two weeks of postnatal life ($r_s=0·65$, $r_s=0·61$, $r_s=0·48$, at intubation, and at weeks one and two, respectively, all $p<0·005$) but not thereafter (all $r_s<0·33$, all $p>0·05$).

SECRETORY COMPONENT ASSOCIATED IMMUNOGLOBULINS AND ANTIBODY IN TRACHEOBRONCHIAL ASPIRATES

Enough of the sample remained from collections at birth and at 4 weeks of age from 12 patients with detectable IgA and IgM at each of these times to measure secretory component associated IgA and IgM concentrations. Secretory component associated IgA and IgM were detectable in all 24 samples. Total concentrations of IgA and IgM correlated significantly with the concentrations of total secretory component associated IgA and IgM, respectively, at each of the sampling times (each $r_s \geqslant 0·64$, each $p<0·01$).

Concentrations of secretory component associated anti-*E coli* antibody were measured in aspirates collected at birth and at four or five weeks after birth from nine patients who had detectable IgA and IgM anti-*E coli* antibody at four or five weeks. At birth none of these nine patients had detectable secretory component associated antibody despite the presence of IgM anti-*E coli* antibody in two of the nine samples tested. Three of the nine samples collected at four or five weeks had detectable amounts of secretory component associated anti-*E coli* antibody.

Discussion

Immunoglobulin in secretions may be acquired from secretion by lymphoid cells resident in mucosa, by transport of systemically produced or acquired immunoglobulin, or a combination of these mechanisms. The results of the longitudinal study of very premature neonates reported here do not determine definitively whether the immunoglobulin or antibody detected in the secretions in prematurity was produced locally by mucosal tissue, or whether it was produced systemically. The decrease in median IgG concentrations in the tracheobronchial aspirates with increasing birth age is consistent with a half life of about four weeks, and thus the IgG may have been from the mother. Concentrations of IgG anti-*E coli* antibody also decreased with increasing postnatal age. There was, however, no correlation between concentrations of IgG and of IgG anti-*E coli* antibody in tracheobronchial aspirates after 2 weeks postnatal age. Thus some IgG antibody may have been produced locally in response to mucosal antigenic stimulation after a postnatal age of 2 weeks.

Support for the concept of local production of immunoglobulin in the mucosa is seen in the rise in concentrations of IgA and IgM with increasing postnatal age, and the increase in the proportion of samples containing IgA and IgM anti-*E coli* antibody with increasing postnatal age. The presence of secretory component associated IgA and IgM in samples collected at birth also suggests maturation of specific mechanisms for acquisition of antibody in secretions. These findings could be the result of the induction of local antibody production at mucosal sites, or of maturation of specific mucosal immunoglobulin transport mechanisms. These mechanisms seem to become active for IgM more rapidly than for IgA, but concentrations of IgA became greater than those for IgM in tracheobronchial secretions by the end of the fifth week of life. IgG concentrations, however, remained four times greater than those of IgA at 5·5 weeks postnatal age.

There were no significant correlations between the concentrations of each immunoglobulin isotype in cord blood samples and in the tracheobronchial aspirates. Similarly the values for tracheobronchial aspirates did not correlate with gestational age. These results suggest that immunoglobulin and antibody present in tracheobronchial secretions is not acquired by passive leakage alone but rather by active mechanisms, which may be dependent on postnatal age rather than gestational age in neonates of less than 30 weeks' gestation. In some neonates, this active process may have begun prenatally, in that tracheobronchial IgG concentrations at birth were greater than concentrations of IgG in cord serum in 10 of 23 neonates studied (43%). Concentrations of IgA in tracheobronchial secretions at birth were also greater than in cord serum in three of 23 neonates (13%). Concentrations of IgM in cord blood were, however, higher than in tracheobronchial fluid in all neonates. Interestingly, concentrations of IgM in tracheobronchial fluid were significantly higher at birth in the seven neonates born after prolonged (>24 hours) rupture of the membranes than in those for whom the duration of rupture of membranes was less than 24 hours before delivery ($p=0·03$, Mann-Whitney U test). In contrast, the median concentration of IgM anti-*E coli* antibody in tracheobronchial fluid from these seven neonates did not differ from the remaining neonates ($p=0·78$). Similarly, there were no significant differences between these two groups in the median concentrations in tracheobronchial fluid of total IgG, total IgA, or IgG, IgA, and IgM anti-*E coli* antibodies. Prolonged rupture of the membranes may therefore act as a stimulus to the appearance of IgM in the respiratory tract, but does not alter concentrations of IgG and IgA and does not lead to production of anti-*E coli* antibody.

IgA antibody to *E coli* in tracheobronchial secretions could originate from maternal breast milk if aspiration of breast milk being given by nasogastric tube feeding occurred. IgA anti-*E coli* antibody was, however, present in samples from seven neonates who had not received breast milk at any time before sample collection. IgA anti-*E coli* antibody was also detectable in three neonates at intubation before any feeds, consistent with the findings of Mellander *et al*, who suggested that IgA antibody in mucosal secretions at birth and in amniotic fluid during pregnancy may be induced by transplacental transfer of maternal IgG anti-idiotype.[19] The three neonates who had secretory component associated anti-*E coli* antibody at four to five weeks postnatally had each received some breast milk, and thus it is not known whether this antibody was produced locally or came from the milk.

The findings of immunoglobulin concentrations in tracheobronchial fluid from very premature neonates in this study are similar to those of Forsyth *et al*, in that there was a slow rise in concentrations of IgA and IgM with increasing postnatal age.[11] In contrast, the rate of rise of concentrations of IgA and IgM was much more rapid in tracheal aspirates from neonates of more than 32 weeks' gestation.[11] In previous studies of neonatal breast milk, we have shown that IgA concentrations rise rapidly in other secretions from neonates born at full term during postnatal life.[12] There was also a rapid rise in concentrations of IgA antibody and secretory component associated antibody in neonatal breast milk to another enteric antigen, β lactoglobulin, during the first two to three weeks of life in neonates born at full term.[12] The results of our study suggest that very premature neonates, although apparently able to acquire mucosal IgA immunoglobulin and specific antibody, do so at a slower rate than gestationally older infants, which suggests slower postnatal maturation of mucosal antibody transport and production than in those born at full term. The presence of secretory component associated immunoglobulin provides presumptive evidence that some of this antibody is produced locally, consistent with the findings of secretory component containing epithelial cells in the respiratory tract during early gestation.[10 20] Paradoxically, however, Takemura and Eishi were not able to find any cells containing immunoglobulin in segmental bronchi in neonates of up to 37 weeks' gestation[10]; thus the origin of the immunoglobulin and antibody reported in this study and in that of Forsyth *et al* remains to be determined.[11]

The production of specific antibody at mucosal surfaces may arise as a result of local antigenic stimulation, or may be a consequence of homing of mucosally associated lymphocytes after exposure to antigens at a distant mucosal site.[21] Only five neonates in this study had clinical and radiological evidence of pulmonary infection at any time during the study period, and *E coli* was not isolated from the bronchial secretions of any infant. These five infants did not have higher concentrations of immunoglobulin or antibody in aspirates than the non-infected neonates.

Previous studies of the microbiological flora in premature neonates (mean gestational age 30 weeks) from this hospital showed that throat swabs from 45% of infants at 17–20 days of age grew *E coli*.[22] *E coli* was also detectable in the faeces of 60% of infants studied at 17–20 days of age.[22] In the study reported here we did not perform routine cultures of tracheobronchial aspirates or faeces at the time of collection of samples for immunoglobulin and antibody estimations. We are therefore not able to say whether the appearance of specific antibody to *E coli* in respiratory tract secretions, if of mucosal origin, is the result of homing of committed lymphocytes from the gastrointestinal tract or is the result of local antigenic stimulation after colonisation of the upper or lower respiratory tract.

The findings of this study suggest that the very premature neonate is able to acquire specific antibody at mucosal surfaces within the first few weeks of postnatal life. The maturation of this process may, however, be slower than that seen in the infant born at full term. It will be necessary to carry out cultures of respiratory tract associated lymphoid cells to determine whether local mucosal production of antibody is possible at this stage of gestation. Further studies will be necessary to see if the therapeutic administration of intravenous immunoglobulin to very premature neonates will affect concentrations of antibodies in the respiratory tracts in these neonates.

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Dexamethasone and infection in preterm babies: a controlled study

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Abstract

To find out if the use of steroids affected the incidence of infection in babies who were nursed in the neonatal intensive care unit for nine weeks or more, 24 preterm babies who had received a three weeks course of dexamethasone (0·6 mg/kg/day, reducing to 0·3 mg/kg/day after a week, and 0·15 mg/kg/day after two weeks) were compared with 18 preterm babies who had not been so treated. No differences were found in the incidence or pattern of septicaemia or other bacteriologically proved infections between the groups. Of 57 episodes of septicaemia, 44 (77%) were caused by coagulase negative staphylococci.

Corticosteroids are being used increasingly to treat bronchopulmonary dysplasia in preterm babies.

The two most serious side effects of treatment with steroids are suppression of the hypothalamic-pituitary-adrenal axis, and predisposition to infection. Neither has been fully evaluated with specific reference to preterm babies, although some information about adrenal suppression is emerging. An association between treatment with steroids and infection is generally assumed in adult patients, but we have not observed either any increase in our overall incidence of infection, or any change in the nature of the infecting organisms, since we started using dexamethasone regularly in the treatment of bronchopulmonary dysplasia. To see if our impression was correct, we compared the incidence and nature of infections in a group of babies treated with steroids and a group of matched controls.

Patients and methods

The study took place between January 1987 and December 1988. The treatment group comprised 24 preterm babies who had received a three week course of dexamethasone and remained on the neonatal unit for at least nine weeks. The course began with 0·6 mg/kg/day, reducing to half this dose at the end of one week, and to a quarter of it at the end of two weeks. The control group comprised 18 preterm babies who were not treated with steroids, but were ventilated for at least two weeks and also remained on the neonatal unit for more than nine weeks. Babies selected to receive dexamethasone were those who were dependent on ventilators because of chronic lung disease, and in whom no appreciable reduction in ventilator settings had been possible over a period of a week. Babies in the control group, although they also had chronic lung disease, were all showing a steady improvement in lung function and so were not considered to need treatment with steroids.

The study was designed to compare the incidence and cause of septicaemia in the two groups during equivalent periods of time. To allow for a secular change in the susceptibility of the babies to infection as a result of increasing maturity and improving health, we compared the postnatal age of the two groups. As the median postnatal age at which steroids were started was 21 days (mean 24 days), we defined three periods of time of three weeks each in which to make both the 'within-group' and the 'between-group' comparisons. These were the first three weeks of life (before steroids in the treatment group), the second three weeks of life (while steroids were being given to the treatment group), and the third three weeks of life (immediately after the steroid treatment had finished). Statistical comparisons were made between corresponding three week periods in the two groups, and between different three week periods in the same group.

All suspected episodes of infection were investigated and documented. Septicaemia was diagnosed when the same organism was isolated from both blood culture bottles, and each isolate showed an identical pattern of antibiotic sensitivity and biochemical profile. Bacteriologically confirmed infections other than septicaemia, such as pneumonia, peritonitis, meningitis, necrotising enterocolitis, and fungal infections were also documented.

An unpaired *t* test was used to assess the significance of differences in the clinical characteristics between the two groups of babies, and the χ^2 test to assess those differences in the incidence of septicaemia and bacteriologically confirmed infections other than septicaemia, both 'within-groups' and 'between-groups'. Permission was obtained from parents in all cases.

Results

Table 1 shows the characteristics of the two groups of babies. For each variable compared the p value is greater than 0·25.

Tables 2 and 3 show the incidence and cause of septicaemic episodes in the treated and control groups. In all the between group comparisons the p values are greater than 0·35, and in all the within group comparisons the p values are greater than 0·08. There is a lower incidence of infection during the third three week period within each group, but this is not significant.

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Table 1 Comparability of the groups

	Group treated with dexamethasone (n=24)		*Control group (n=18)*	
	Mean (SD)	*Median*	*Mean (SD)*	*Median*
Gestational age (weeks)	27·7 (2·0)	27·5	27·2 (1·9)	27·0
Birth weight (g)	1041 (292)	978	1032 (196)	1073
Temperature on admission (°C)	35·8 (1·0)	36·0	35·5 (0·7)	35·5
Apgar score at one minute	4·0 (2·4)	4·0	3·7 (2·6)	3·0
Apgar score at five minutes	7·8 (1·6)	8·0	7·5 (1·3)	7·0
No of days receiving assisted ventilation	26·5 (16·8)	24·5	29·1 (15·6)	24·0
No of days dependent on supplementary oxygen	69·9 (44·4)	54·0	58·3 (27·5)	51·5
Male:female ratio	13:11		8:10	

Table 2 Incidence of septicaemia in the two groups in each of the three time periods

	Group treated with dexamethasone (n=24)			*Control group (n=18)*		
	Weeks 1–3	*Weeks 4–6*	*Weeks 5–9*	*Weeks 1–3*	*Weeks 4–6*	*Weeks 5–9*
No (%) who had two episodes of septicaemia	1 (4)	1 (4)	1 (4)	3 (17)	1 (6)	0
No (%) who had one episode of septicaemia	11 (46)	10 (42)	4 (17)	6 (33)	9 (50)	4 (22)
No (%) who did not develop septicaemia	12 (50)	13 (54)	19 (79)	9 (50)	8 (44)	14 (78)
Total No of episodes of septicaemia	13	12	6	12	11	4

Table 3 Number (%) of organisms isolated from blood in 58 episodes of septicaemia

	Group treated with dexamethasone (n=24)			*Control group (n=18)*		
	Weeks 1–3	*Weeks 4–6*	*Weeks 5–9*	*Weeks 1–3*	*Weeks 4–6*	*Weeks 5–9*
Total No of episodes of septicaemia	13	12	6	12	11	4
Organism:						
Coagulase negative staphylococcus	8 (62)	11 (92)	5 (83)	9 (75)	9 (82)	2 (50)
Streptococcus faecalis	2 (15)	0	0	0	1 (9)	0
Staphylococcus aureus	1 (8)	0	0	0	1 (9)	0
Bacteroides spp	1 (8)	0	0	0	0	0
Acinetobacter spp	0	0	1 (17)	1 (8)	0	1 (25)
Neisseria gonorrhoeae	0	0	0	1 (8)	0	0
Haemophilus influenzae	0	0	0	1 (8)	0	0
Candida spp	1 (8)	1 (8)	0	0	0	1 (25)

Coagulase negative staphylococci were the organisms most frequently isolated, causing 44 of the 57 (77%) of the episodes of septicaemia. All the episodes occurred when at least one intravascular cannula was in place.

When bacteriologically confirmed infections other than septicaemia were included, the difference in the incidence of infection 'between-groups' and 'within-groups' was not significant ($p>0·35$ and $p>0·06$, respectively). The third three week period in both groups again tended to have a lower incidence of infection when compared with the preceding six weeks. Pneumonia confirmed by bacteriology ($n=6$) was the second commonest infection after septicaemia. Fungal infection ($n=1$), necrotising enterocolitis ($n=2$), peritonitis ($n=1$), and cytomegalovirus infection acquired from transfusion ($n=1$) were evenly distributed between the two groups.

Discussion

The mechanisms by which steroids might predispose to infection are not clear. Recent work points to an effect on leucocyte kinetics causing eosinopenia, lymphocytopenia, monocytopenia, and neutrophilia.[1] The neutrophilia is caused by a shift of neutrophils from the marginal pool into the blood stream and is accompanied by a blunting of the chemotactic response.

The extent to which steroids truly predispose to infection is, however, overestimated.[1 2] Several studies have shown little or no increase in the incidence of infection among steroid treated adults compared with controls,[1–4] as did the only single small study in neonates that we could find.[5]

One explanation for the apparent increase in infection in patients treated with steroids is the influence of the underlying disease or state of the patient. Preterm babies are all immunocompromised to some extent, and have poor neutrophil chemotaxis, so that steroids may impose little further increase in risk.

Another factor is that many infections in both children and adults treated with steroids are reactivations of latent infection, probably the result of lymphopenia and monocytopenia.[1] In newborn infants, infections are the result of new bacterial invasion, often directly into the blood stream through an intravascular line; this process is uninfluenced by steroids.[1]

Also relevant to the results of our study is the fact that the anti-inflammatory potency of a particular steroid has little influence on its immunosuppressive effect. Dexamethasone, which is one of the most potent corticosteroids, has comparatively little immunosuppressive property.[6]

The results of our study show that the use of dexamethasone in the treatment of bronchopulmonary dysplasia is not associated with an appreciable increase in the incidence of infection, although the possibility of a Type II error prevents us from ruling out a small effect. Steroids should not be withheld for fear of predisposing to infection.

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Neonatal pneumomediastinum with isolated mitral obstruction

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Abstract

While mediastinal air accumulation in the ventilated newborn is usually a benign entity, tension pneumomediastinum can embarrass both respiratory and cardiac function. A case of isolated left ventricular inflow obstruction caused by a pneumomediastinum in a premature infant is described that resolved with high frequency ventilation. The development of pneumomediastinum and haemodynamic compromise in a premature infant warrants echocardiographic examination.

Intrathoracic air accumulation is a common complication of mechanical ventilation in the newborn intensive care unit. The clinical consequences of pneumomediastinum and pulmonary interstitial emphysema in the premature infant have been well described.[1 2] Pneumomediastinum is a less commonly encountered result of barotrauma, often having minimal pulmonary or cardiac consequences. We here describe a case of pneumomediastinum in a low birthweight premature infant that caused haemodynamic compromise secondary to isolated left ventricular inflow obstruction. High frequency ventilation was successfully used to decompress the mediastinum and restore normal cardiac function.

Case report

A boy, weighing 750 g, was born vaginally at 25 weeks' gestation to a 23 year old woman (gravida 4, para 3) with an uncomplicated prenatal course. Labour was spontaneous and Apgar scores were 4 at 1 minute and 5 at 5 minutes with immediate intubation in the delivery room. During the first 12 hours of life, the patient required increasing ventilator rates and pressures secondary to severe hyaline membrane disease. A small patent arterial duct was diagnosed by echocardiography at 12 hours of age and indomethacin treatment was instituted.

During the next 12 hours, the ventilatory peak pressures and rates were modestly weaned. The infant's chest radiograph, however, showed signs of worsening disease with the appearance of interstitial emphysema. During the second day of life there were increasing ventilator requirements. Peak ventilatory pressures of 30 cm H_2O (mean airway pressure of 17 cm H_2O) and rates of 70 breaths/minute and 100% oxygen were needed to maintain a pH of 7·24, a carbon dioxide arterial pressure of 7·8 kPa and an oxygen arterial pressure of 7·8 kPa.

On day three of life, the patient continued to require maximal respiratory support. A chest radiograph showed free air in the mediastinum and increasing opacity of the lung fields (fig 1a). Subcutaneous emphysema was also noted. Repeat echocardiography was performed to assess the efficacy of the indomethacin treatment. Pulsed Doppler examination confirmed that the arterial duct had closed. The four chamber view, however, showed a large mass like effect superior and posterior to the left atrium (fig 1b). Pulsed Doppler examination showed evidence of inflow obstruction at the mitral valve with turbulent flow and a peak inflow velocity of 100 cm/second (fig 1c). Simultaneous tricuspid inflow was laminar with a peak inflow velocity of 20 cm/second (fig 1d). Postulating that a tension pneumomediastinum was compromising mitral inflow and possibly contributing to pulmonary oedema, strategies to decompress the mediastinum were considered. In view of the infant's precarious condition, surgical decompression was thought to be too risky. High frequency jet ventilation at a rate of 420 breaths/minute was instituted and a reduction of mean airway pressure from 17 to 8 cm/H_2O was effected.

Over the next 12 hours, the infant's chest radiograph showed resolution of mediastinal air (fig 2a) with concurrent improvement in arterial was tested at tenfold dilutions ranging from $1/10^3$ to $1/10^8$ on each plate.[13] With this assay IgG was detectable to 2×10^{-7} g/l, IgA to 4×10^{-7} g/l, and IgM to 3×10^{-7} g/l.

Enzyme immunoassays for the estimation of total concentrations of IgG, IgA, and IgM in tracheobronchial secretions were carried out as previously described.[12] All samples were tested in duplicate at three dilutions. A reference preparation of pooled multiple tracheobronchial aspirates from two patients aged 6 years and 8 years (who had permanent tracheostomies for management of high spinal injuries) was included on each assay plate in fivefold dilutions ranging from 1/500 to 1/1562500 so that a standard curve for the determination of relative units for each test sample could be derived. The concentrations of IgG, IgA, and IgM/mg total protein in this reference preparation were 58·2 μg, 159·1 μg, and 20·9 μg, respectively. The lower limit of sensitivity was $3{\cdot}9\times10^{-7}$g/l, $3{\cdot}6\times10^{-7}$g/l, and $1{\cdot}4\times10^{-7}$g/l for IgG, IgA, and IgM, respectively.

Enzyme immunoassays for the determination of secretory component associated immunoglobin were carried out by modifications of the above assays in which a murine monoclonal antisecretory component antibody (Australian Monoclonal Developments, Sydney) was used to detect secretory component associated IgM or IgA bound to the solid phase by affinity isolated anti-IgM or anti-IgA precoating antibody (Tago, Burlingame, California). Affinity isolated peroxidase conjugated antimouse IgG was used to develop the substrate reaction.

ANTIBODY TO POOLED *E COLI* ANTIGENS

Enzyme immunoassays were used to measure concentrations of IgG, IgA, and IgM antibodies specific to *E coli* polysaccharide in all samples of tracheobronchial fluid and cord serum. A pool of eight strains of *E coli* representing strains found most frequently in faecal isolates or diseases (serotypes 01, 02, 04, 06, 07, 016, 018, and 075) was prepared by a heat extraction technique.[14 15]

Ninety six well microtitre plates (Nunc) were incubated with 100 μl of *E coli* antigen in 0·05 M bicarbonate buffer (pH 9·5; final concentration 0·09 μg *E coli* antigen/ml) in each test well for two hours at 37°C and then overnight at 4°C. Residual binding sites were blocked with 50 μ/well 2·5% skim milk powder in 0·05 M bicarbonate buffer (pH 9·5) for two hours at 37°C.[16] After washing, 50 μl/well of sample diluted in phosphate buffered saline containing 0·05% Tween 20 (PBS-T) (pH 7·2) and containing 2·5% skimmed milk powder (SMP) was added and incubated for two hours at 37°C. After washing, 50 μl of a 1:1000 dilution of peroxidase conjugated, affinity isolated, goat antihuman IgG, or IgA, or IgM (Tago), in PBS-T/SMP was added and incubated for two hours at 37°C. The plates were then washed and 50 μl of orthophenylene diamine substrate was added. The reaction was stopped after incubation for 15 minutes in the dark by adding 25 μl of 8N hydrochloric acid solution to each well. Absorbance values were read at 492 nm using a Titertek Multiscan (Flow Laboratories).

All samples were tested in duplicate and at three dilutions ranging from 1/10 to 1/250 for tracheobronchial fluid samples, and from 1/10 to 1/1250 for cord samples. The pooled human reference serum[13] and the tracheobronchial fluid reference preparation were each included in six serial dilutions on every test plate. Each test sample was quantitated by reading from interpolated points generated from the linear portion of the appropriate reference standard curve. The concentration of anti-*E coli* antibodies in the reference preparations was estimated using a modification of a method previously described.[17] The pooled reference serum contained $0{\cdot}42\times10^{-3}$g IgA anti-*E coli* antibody/l, $3{\cdot}43\times10^{-3}$g IgG antibody/l, and $6{\cdot}98\times10^{-3}$g IgM antibody/l, whereas the tracheobronchial fluid reference preparation contained 31·01 ng IgA anti-*E coli* antibody/mg total protein, 6·25 ng IgG antibody/mg total protein, and 51·14 ng IgM antibody/mg total protein.

Specificity of the assays for IgG, IgA, and IgM antibodies to the *E coli* antigens was illustrated by appropriate liquid phase absorption experiments using the reference sample preparations and the pooled *E coli* antigen preparation. The lower limit of detection was taken at optical density values of more than twice the background (sample free) optical density readings. The lower limit of IgG, IgA, and IgM anti-*E coli* antibody detection (g/l) for the serum reference preparation was $1{\cdot}2\times10^{-7}$, $1{\cdot}0\times10^{-7}$, and $0{\cdot}9\times10^{-7}$, respectively, and $0{\cdot}3\times10^{-7}$, $0{\cdot}1\times10^{-7}$, and $0{\cdot}1\times10^{-7}$ for the tracheobronchial fluid reference preparation.

Secretory component associated antibody to the pooled *E coli* antigens was determined by appropriate modification of a previously described assay.[12]

TOTAL PROTEIN QUANTITATION

Measurements of total protein concentration were made by the method of Lowry *et al.*[18] To correct for any errors introduced by sample dilution during endotracheal tube aspiration, the concentration of tracheobronchial immunoglobulin or specific antibody in samples was expressed as a ratio of μg of immunoglobulin or ng of antibody/mg of total protein in the sample.

STATISTICAL ANALYSIS AND ETHICS

Results in the tables are given as medians for all samples. As age increased after birth fewer neonates remained intubated. Statistical comparisons of results at differing postnatal ages were made using paired samples only. Analyses of immunoglobulin concentrations in paired samples were done by the Wilcoxon matched pairs signed rank test and the Spearman rank order correlation analysis. Longitudinal comparisons of proportions of study samples with detectable anti-*E coli* antibody were done with the McNemar test for the significance of changes.

The study was approved by the institutional human ethics committees for each of the three participating neonatal intensive care units. Informed written consent was obtained from the parents of all neonates before enrolment in the study.

Results

TOTAL IGG, IGA, AND IGM CONCENTRATIONS

All samples of cord serum contained IgG, IgA, and IgM. The median concentrations for IgG, IgA, and IgM were 4·87 g/l (range 1·82–9·52 g/l), 0·004 g/l (range 0·001–0·04 g/l), and 0·07 g/l (range 0·04–0·58 g/l).

A total of 144 tracheobronchial aspirates from 33 premature neonates were collected and analysed. The median immunoglobulin concentrations/mg total protein in tracheobronchial fluid samples at each postnatal collection time are shown in table 1. All aspirates contained IgG, with the highest concentrations present at birth (table 1). Two weeks later, the median IgG concentration had decreased significantly (Wilcoxon matched pairs signed rank test, 24 pairs, $p<0\cdot03$), and the median IgG concentration at 5·5 weeks remained significantly lower than at the time of perinatal intubation (10 pairs, $p<0\cdot02$).

The median IgA concentrations in the tracheobronchial aspirates are shown in table 1. IgA was detectable in 26 of the 28 aspirates collected at birth (93%), although the median IgA concentration was low (0·7 μg/mg total protein). The median IgA concentration remained low during the first three weeks, and then showed a progressive increase (Wilcoxon: between three and four weeks after birth, 16 pairs, $p<0\cdot04$; between 3 and 5·5 weeks after birth, nine pairs, $p<0\cdot03$). The median concentration of IgA in tracheobronchial aspirates at 5·5 weeks of age was 5·8 μg/mg total protein (range 0·3–13·0 μg/mg total protein, n=10).

IgM was detectable in samples from 22 of the 28 neonates studied at birth (79%). Within the first week the median IgM concentration increased significantly (Wilcoxon; 24 pairs, $p<0\cdot001$), but there was no further significant change thereafter.

The ratio of the median concentration of total IgA: total IgM in tracheobronchial aspirates increased significantly after the second week (Wilcoxon: week 2 to week 3, 17 pairs, $p<0\cdot01$), reaching a value of 1·0 at about five weeks (Wilcoxon: week 2 to 5·5, eight pairs, $p<0\cdot02$).

Using the Spearman rank order correlation analysis, there were no significant correlations between IgG, IgA, and IgM concentrations in samples of cord serum (mg/100 ml) and in tracheobronchial fluids (μg/mg total protein) collected at the time of perinatal intubation (n=23, each $r_s<0\cdot38$, each $p>0\cdot05$). Similarly, there were no significant correlations between concentrations of tracheobronchial IgG, IgA, and IgM (μg/mg total protein) at delivery and the gestational age at birth (n=28, each $r_s<0\cdot21$, each $p>0\cdot14$).

ANTIBODY TO POOLED *E COLI* ANTIGENS

The median concentrations of IgG, IgA, and IgM antibodies to the pooled *E coli* antigens in cord samples were $0\cdot91\times10^{-3}$g/l (range 0·10–5·08), 0×10^{-3}g/l (range 0–0·01), and $0\cdot03\times10^{-3}$g/l (range 0–0·46) respectively.

The median concentrations of anti-*E coli* antibody in tracheobronchial fluid at differing postnatal ages are shown in table 2. Table 3 shows the percentage of analysed tracheobronchial fluid samples with detectable anti-*E coli* antibody within the three isotypes IgG, IgA, and IgM at differing postnatal ages.

Almost all samples contained IgG anti-*E coli* antibody. At the time of intubation, IgG anti-*E coli* antibody was detected in 25 of 28 samples (89%) and the median concentration was 19·1

Table 1 Median (range) concentrations of IgG, IgA, and IgM in samples of tracheobronchial fluid (μg/mg total protein)

	Birth (n=28)	*Week 1 (n=27)*	*Week 2 (n=29)*	*Week 3 (n=21)*	*Week 4 (n=18)*	*Week 5 (n=12)*	*Week 5·5 (n=10)*
IgG	61·5 (25·1–381·5)	57·8 (13·3–238·0)	46·1 (16·4–156·9)	37·1 (12·2–90·5)	27·1 (11·8–81·5)	22·9 (13·4–47·5)	22·6 (5·6–33·5)
IgA	0·7 (0·0–3·5)	0·9 (0·0–6·94)	0·6 (0·0–5·8)	0·7 (0·2–8·4)	1·4 (0·1–10·5)	3·4 (0·3–18·1)	5·8 (0·3–13·0)
IgM	0·6 (0·0–4·3)	2·1 (0·2–21·6)	2·1 (0·2–13·1)	2·6 (0·3–38·8)	2·8 (0·3–8·2)	3·3 (0·2–15·0)	2·9 (0·2–8·5)

Table 2 Median (range) concentrations of IgG, IgA, and IgM anti-E coli antibody in samples of tracheobronchial fluid (ng/mg total protein)

	Birth (n=28)	*Week 1 (n=27)*	*Week 2 (n=29)*	*Week 3 (n=21)*	*Week 4 (n=18)*	*Week 5 (n=12)*	*Week 5·5 (n=10)*
IgG	19·1 (0–189·5)	8·7 (0–218·7)	6·2 (0–72·9)	5·6 (0–34·2)	3·8 (0–22·5)	2·9 (0–16·8)	3·2 (0–16·4)
IgA	0·0 (0–1·6)	0 (0–1·2)	0 (0–10·5)	0 (0–0·6)	0 (0–0·5)	0 (0–19·8)	0·1 (0–54·6)
IgM	0 (0–7·7)	0 (0–5·7)	0·3 (0–8·9)	0·3 (0–1·6)	0·3 (0–1·0)	0·2 (0–2·1)	0·2 (0–2·6)

Table 3 Percentage of tracheobronchial aspirates with detectable amounts of IgG, IgA, and IgM anti-E coli antibody at increasing postnatal ages

	Birth (n=28)	*Week 1 (n=27)*	*Week 2 (n=29)*	*Week 3 (n=21)*	*Week 4 (n=18)*	*Week 5 (n=12)*	*Week 5·5 (n=10)*
IgG	90·2	81·4	83·0	76·4	89·1	75·2	70·3
IgA	11·1	18·5	21·4	23·8	33·3	41·7	50·0
IgM	30·4	40·7	62·1	66·7	61·1	58·3	60·0

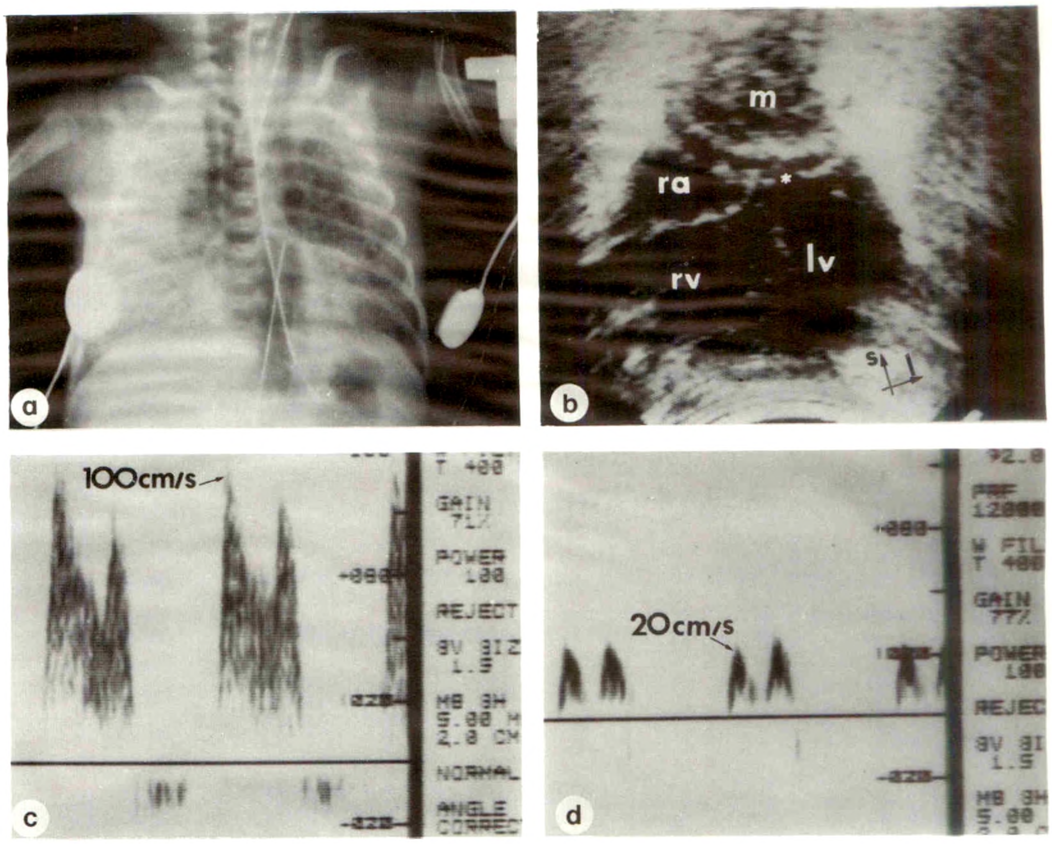

Figure 1a, Infant's chest radiograph showing mediastinal and subcutaneous air accumulation as well as opacified lung fields. 1b, Echocardiogram (four chamber view) showing large mass effect (m) superior to the left atrium. Asterisk indicates area of mitral valve commissural opening; ra, right atrium; rv, right ventricle; lv, left ventricle; s, superior; and l, left. 1c, Pulsed Doppler examination of mitral inflow before high frequency jet ventilation treatment. Note turbulent flow as well as abnormal 100 cm/second peak inflow velocity. 1d, Pulsed Doppler examination of tricuspid inflow before high frequency jet ventilation showing normal laminar flow and peak inflow velocity of 20 cm/second.

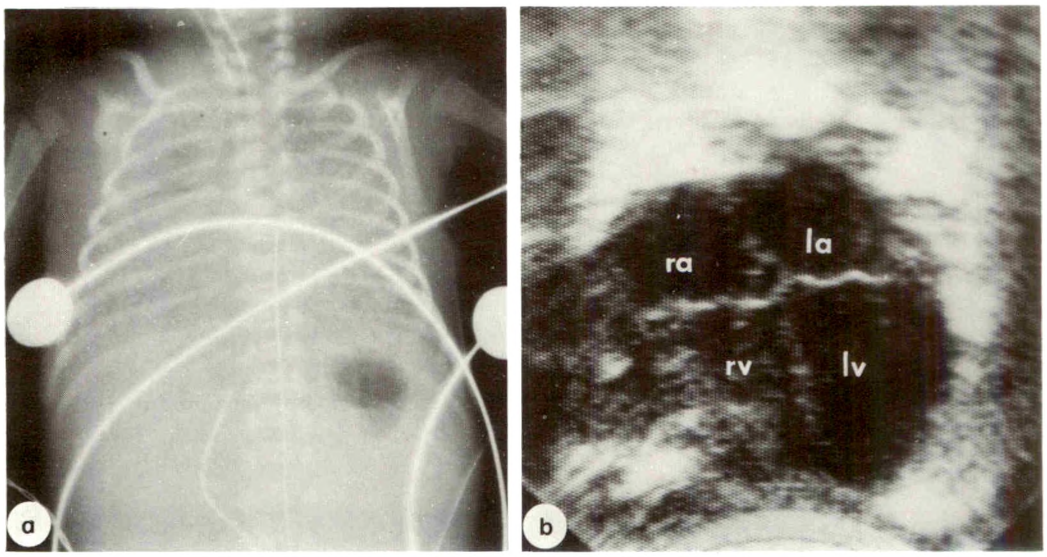

Figure 2a, Infant's chest radiograph after high frequency jet ventilation showing resolution of mediastinal air. 2b, Repeat echocardiogram (four chamber view) after high frequency jet ventilation showing resolution of mass effect on left atrium; ra, right atrium; rv, right ventricle; lv, left ventricle; la, left atrium.

blood gases. Repeat echocardiography showed resolution of the mass effect impinging on the left atrium (fig 2b) and normal mitral inflow.

Discussion

The clinical consequences of intrathoracic air accumulation in the neonate secondary to mechanical ventilation have been well described.[1 2] Air trapped in the pleura, lung parenchyma, or mediastinum, especially if under tension, can embarrass respiratory and cardiac function.

A tension pneumomediastinum can interfere with the normal intrathoracic pressure associations, compromising normal venous return patterns to the right and left sides of the heart.[3] This may be reflected in low right and left ventricular inflow velocities on pulsed Doppler examination. Most cases of pneumomediastinum in the neonate result in anterior air accumulation with negligible cardiac or respiratory sequelae. A large isolated posterior mediastinal air accumulation is much less common, but may also lead to respiratory decompensation as has recently been described.[4]

The above case illustrates a previously undescribed cardiac side effect of pneumomediastinum in the neonate. The echocardiogram showed an air filled mass compressing the superior, posterior left atrial wall impinging upon the mitral valve. In contrast with the low velocity signal that would have been obtained had the systemic or pulmonary veins been obstructed, the pulsed Doppler pattern confirmed obstruction at the mitral valve level. Venous return through the tricuspid valve, however, as evidenced by Doppler flow, was normal. This localised cardiac effect is postulated to have resulted from the regional differences of air accumulation in the posterior mediastinum.

The physiological consequences of acute mitral valve obstruction are varied depending on the severity. Both pulmonary oedema and low cardiac output are possible sequelae. In our case, the increased opacity of the lung fields on the chest radiograph paralleled the development of mitral obstruction. It is not clear, however, whether the change in the chest radiograph reflected pulmonary oedema, worsening lung disease, or both. Similarly, although cardiac output was compromised during this time, the relative contribution of mitral obstruction is difficult to quantify. Clinical improvement rapidly followed resolution of the pneumomediastinum.

Surgical decompression has been described as the treatment of choice for tension pneumomediastinum.[2 4] High frequency ventilation, however, has been recently utilised in the neonate to treat life threatening pulmonary air leaks[5]; this was effectively employed to decrease the air leakage and decompress the mediastinum in our patient. Normal left ventricular venous inflow was demonstrated after resolution of the mediastinal air.

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CURRENT TOPIC

Prenatal diagnosis of enzyme defects

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About 250 enzyme deficiencies have been demonstrated in the 750 or so recognised human recessive disorders.[1] Approximately 100 of these enzymopathies can be diagnosed prenatally. Undoubtedly this number will increase as more methods are developed for the detection of enzymic defects in fetal samples (particularly in chorionic villus samples obtained in the first trimester). There are two main limitations to the application of the enzyme assay procedures used in postnatal diagnosis to fetal samples. Firstly the amount of material available for investigation is very small. Therefore the enzymic assay procedure has to be very sensitive, which may necessitate modification of postnatal methods. Secondly the enzyme under investigation may not be expressed in fetal samples that are readily accessible, for example, chorionic villi or cultured amniotic cells, because it is tissue specific. Although sampling of some specialised tissues is possible, the enzyme may still not be expressed at a sufficiently early stage of gestation to allow diagnosis and termination. These problems can, in principle, be overcome by the use of recombinant DNA technology, which detects or tracks mutations in genomic DNA. As this does not require expression of the enzyme protein, it can be applied to any nucleated cell. Currently, however, DNA probes are being used for the prenatal diagnosis of a very small number of enzymic defects (see appendix) and modifications of conventional assay procedures are used predominantly. The expansion of DNA analysis depends upon progress in cloning genes encoding enzymes and in elucidation of the molecular genetical basis of enzymopathies. This article will review the range of enzymic defects that can be detected prenatally and discuss how the method of detection depends upon the molecular basis of the defect, the tissue and subcellular site of action of the enzyme, and the nature of the fetal biopsy available.

Indications for prenatal diagnosis of an enzyme defect

Prenatal diagnosis of an enzymic defect is generally only carried out for pregnancies known to be at high risk for a severe disorder for which there is no effective treatment. Most requests for prenatal monitoring of a pregnancy will be from parents who have previously had a child with an enzyme deficiency and for whom termination of the pregnancy is acceptable. In the case of X linked disorders, a woman who has an affected brother or whose sister has given birth to an affected child may also request testing. The reliability of the test is crucial and these tests should only be carried out in experienced laboratories. The precise diagnosis of the enzymic defect in the index case is a prerequisite for undertaking such investigations. Because it is selective, prenatal diagnosis of enzymic defects is a highly effective preventative measure. Experience from our laboratory (unpublished observations) and others,[2–4] shows that 18–25% of the fetuses tested are affected with the suspected defect, very close to the expected 25%. If a pregnancy is terminated as a result of testing, it is important to confirm the diagnosis on the aborted fetus to assess the efficiency of the diagnostic procedure and to reassure the parents. If possible a cell culture should be established and specimens preserved from the fetus for future reference. Similarly prediction of an unaffected fetus can be confirmed postnatally by biochemical tests but this is usually only carried out now at the parents' request or if there is any concern over the health of the baby.

Molecular basis and heterogeneity of enzymic defects

A complex sequence of events takes place before an enzyme can function correctly in vivo. The gene encoding the enzyme has to be transcribed in a controlled manner and the RNA transcript edited before translation into a precursor enzymic protein. Subsequently most newly synthesised proteins undergo some form of post-translational modification or maturation before or during transport to a specific intracellular or extracellular site of action. The mature enzymic protein may require a cofactor or association with one or more proteins, encoded by other genes, to be fully active. The complexity of the biosynthesis and intracellular localisation of an enzyme affords many opportunities for genetic errors. Accordingly an enzyme deficiency leading to a characteristic pathological phenotype may be due to several different underlying molecular defects. In many disorders there is residual enzymic activity, the amount of which may be related to the age of onset or severity of the disease. It is generally possible to differentiate prenatally among the variant forms of a disease due to different mutations if the molecular basis of the defect has been clearly

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established in the index case. However this will often involve a more elaborate detection procedure than a simple assay of enzymic activity in a crude tissue homogenate.

Fetal sampling

The demonstration of an enzymic defect requires removal of fetal material for biochemical analysis. This is carried out under the guidance of ultrasonography or by fetoscopy.

CHORIONIC VILLUS SAMPLING

Chorionic villus sampling without ultrasound was first used in China in 1975 for diagnostic fetal sexing,[5] but the first measurement of enzymic activities in chorionic villi was in 1982.[6] Currently chorionic villus sampling is carried out with the assistance of ultrasonography by transcervical aspiration[7] or biopsy with forceps[8] between eight and 12 weeks' gestation but optimally between nine and 10 weeks. Transabdominal aspiration is an alternative that decreases the risk of infection and permits sampling between 12 and 14 weeks obviating waiting for amniocentesis at 16 weeks or later. The risk to pregnancy of chorionic villus sampling is 2–4%, about 2% higher than that for spontaneous abortion and about twice that for amniocentesis.[9–11] As more centres gain experience of chorionic villus sampling this risk may decrease. Over the last three years chorionic villus sampling has become more widespread and popular because of the advantage of early diagnosis. This has stimulated the development of suitable enzyme assay procedures. A major problem is the contamination of the chorionic villi biopsy specimen with maternal material. The sample should be examined under a low power dissecting microscope and maternal material, mainly decidua, removed. A typical sample of 10–40 mg wet weight will provide enough material for the assay of the enzyme under investigation and a reference enzyme and for the measurement of protein. All the enzymic defects that have been detected in cultured amniotic fluid cells can be detected in chorionic villi cells. Most enzymes can be assayed directly in a homogenate of the biopsy specimen enabling a diagnosis to be made within one to three days of sampling. For a few enzymes, whose activity is very low in normal chorionic villi, it is necessary to obtain a culture of cells from the sample,[2 3 12] thereby delaying the result for two to three weeks. In contrast the detection of a deficiency of arylsulphatase A or B for metachromatic leucodystrophy and Maroteaux-Lamy disease respectively is difficult in chorionic villi because of the very high activity of a competing enzyme arylsulphatase C in trophoblasts.[13] Until recently the secondary multiple lysosomal enzyme deficiency in mucolipidosis II (I-cell) and mucolipidosis III (pseudo-Hurler dystrophy) could only be demonstrated in cultured chorionic villi cells. Ben Yoseph *et al* have shown that it is possible to detect the primary defect of N-acetylglucosamine 1-phosphotransferase directly in chorionic villi obtained at nine weeks.[14]

Fresh intact chorionic villi can also be used to study enzymic activities by the uptake and utilisation of radioactively labelled metabolic precursors and for the extraction of DNA for diagnosis using recombinant DNA techniques (see below). For pregnancies at high risk chorionic villus sampling has become the method of choice because most physicians and parents consider the advantage of early diagnosis to compensate for the increased risk. Transabdominal removal of chorionic villi from the placenta has been successfully carried out in the second and third trimester for diagnosis of genetic abnormalities by direct karyotyping without cell culture.[15] Such samples could also be used for enzymic assays.

AMNIOCENTESIS

Enzymic defects can be investigated both in amniotic fluid by detection of specific metabolites and in cultured amniotic fluid cells by enzyme assay or incorporation studies. Amniocentesis under the guidance of ultrasonography is a well established and reliable procedure with a risk of miscarriage of about 0·5% in centres with experienced staff.[16] A sample of 10–20 ml of amniotic fluid is removed by transabdominal puncture at 15–16 weeks' gestation, the optimal time with regard to cell number and viability. The amniotic fluid and cells are separated and a culture initiated of the cells. Amniotic cells cannot be used directly because many are non-viable and in various states of decay. It can take up to six weeks to obtain sufficient viable cells for some enzyme determinations but three to four weeks is more usual. Experience of cell culture, especially of amniotic cells, is necessary to assess the state of readiness of the cultures for biochemical analysis.[17] Appropriate controls must always be available and analysed at the same time. A wide range of enzymic defects has been diagnosed by analysis of amniotic material. Although most of these disorders can now be diagnosed on chorionic villus samples, amniocentesis will still be widely used until more centres gain experience of chorionic villus sampling, for late presentations and because of the lower risk associated. In some situations a diagnosis made in the first trimester by chorionic villus sampling, particularly of an unaffected fetus, is confirmed by amniocentesis. DNA can be extracted from cultured amniotic fluid cells for analysis.

FETAL BLOOD AND TISSUES

Some enzymopathies cannot be diagnosed in chorionic villi or cultured cells from chorionic villi or amniotic fluid because the affected enzyme is not expressed in these cells. Examples are the liver-specific enzymes of the urea cycle. Some specific fetal tissues can be obtained for biochemical analysis but considerable experience and expertise are needed for these procedures.[18]

Fetal blood may be obtained by puncture of vessels in the fetal heart, umbilical cord, or chorionic plate under the guidance of ultrasonography or by fetoscopy. Currently cordocentesis guided by ultrasound at 18–20 weeks' gestation is recommended.[19 20] Immunodeficiencies due to enzyme defects have been diagnosed by measurement of enzymes in fetal erythrocytes and/or metabolites in blood.[21] Adenosine deaminase, the enzyme involved in about 20% of cases of severe combined immunodeficiency, can also be measured in chorionic villi.[22] Sometimes relatively high residual activity is found and the diagnosis has to be confirmed on fetal blood. A deficiency of tetrahydrobiopterin has been diagnosed by assaying the enzyme 6 pyruvoyl tetrahydropterin synthase in fetal erythrocytes, together with demonstration of pterins in amniotic fluid.[23] White blood cells are used extensively for the postnatal diagnosis of enzyme defects and fetal white blood cells could be used for the same tests. Very little material is required and the procedure would be rapid because cell culture is not necessary. The risk and lateness for an elective abortion preclude this approach except under special circumstances, such as a failed amniocentesis or a very late request for prenatal testing of a high risk pregnancy.

Fetal liver biopsies taken after 19 weeks' gestation by aspiration under fetoscopic or ultrasound guidance have been used for the prenatal diagnosis of several liver specific enzyme defects: ornithine carbamoyltransferase,[24] carbamoyl phosphate synthase I,[25] glucose-6-phosphatase type Ia (von Gierke's disease),[26] and the peroxisomal disorder, hyperoxaluria type I due to alanine:glyoxylate aminotransferase deficiency.[27] Ornithine carbamoyltransferase deficiency can now be diagnosed in chorionic villus samples using a gene specific DNA probe.[28] It is predicted that the technique of liver biopsy for enzymic defects will become obsolete as more liver enzyme probes become available.

Fetal skin biopsies may be obtained by fetoscopy under real time ultrasound guidance at 18–21 weeks' gestation.[29] Most skin disorders are diagnosed prenatally by histological and ultrastructural studies of the fetal skin biopsy specimens because the underlying molecular defect is unknown. Where an enzyme is involved, as in Ehlers-Danlos syndrome type VI, Fabry's disease, Refsum's disease, or X linked ichthyosis diagnosis can be made on chorionic villi or cultured amniotic fluid cells.

Methods of detecting an enzymic deficiency

DIRECT ASSAY

The simplest and most direct way of demonstrating an enzyme defect is to assay the enzymic activity in a crude tissue homogenate using a specific substrate under optimal conditions. For many enzymes it is possible to use synthetic substrates, which combine ease of measurement with the greater sensitivity necessary for fetal samples—for example, the fluorigenic 4-methylumbelliferyl conjugates widely used for the assay of lysosomal hydrolases. For other enzymes it is essential to use a natural substrate because of the strict specificity of the enzyme or because it discriminates among closely related activities.[30] In some assays the product is measured indirectly by a coupled enzymic reaction—for example, the release of glucose by debranching enzyme in amniocytes.[31] All soluble enzymes can be assayed directly in tissue homogenates but membrane-associated enzymes have to be released by a detergent, which may also act to solubilise a lipophilic substrate.

The amount of enzymic activity is related either to soluble protein or to the activity of a comparable unaffected enzyme with the same subcellular location (or more rarely to a number of cells). A complete deficiency of the enzyme is usually obvious but it is essential to assay material from the index case and a control at the same time. The reference range for normal controls should be established before offering the test. If the activity is well below the normal range but greater than that in the index case it is important to exclude certain situations before concluding that the fetus is a carrier of a recessive trait and therefore unaffected. The low residual activity could be due to a mutant enzyme or to a genetically determined pseudo deficiency, both of which can generally be recognised by characterisation of the index case and family. Another possibility is that a related but unaffected enzyme also acts on the same substrate. In Tay-Sachs disease, which is due to a deficiency of hexosaminidase A, there is considerable residual hexosaminidase activity in chorionic villi or cultured amniotic cells due to the isoenzyme hexosaminidase B. The true deficiency of hexosaminidase A can be measured by a differential assay based on a difference in thermal stability of the two isoenzymes,[32] by the use of a specific substrate,[33] or by chromatographic or electrophoretic separation of the two isoenzymes.[34] In the AB variant of GM_2 gangliosidosis there is normal hexosaminidase A and B activity towards synthetic substrates but gangliosides are not catabolised in vivo because of the deficiency of an activator protein. This situation can be diagnosed prenatally but emphasises the need for clear diagnosis of the index case.[35]

The diagnosis of primary hyperoxaluria type I by the assay of the peroxisomal enzyme, alanine:glyoxylate aminotransferase in fetal liver homogenates, is complicated by the presence of the cytosolic enzyme, glutamate: glyoxylate aminotransferase, which has activity towards the same substrate.[27] As there is not enough material in such a biopsy specimen to measure both enzymes, a correction based on the activity of the competing enzyme in control fetal livers is made. Subcellular fractionation is an alternative solution to the problem of isoenzymes in different subcellular compartments. The maintenance of the integrity of an organelle is necessary for the detection of some enzymic defects. The activity of some enzymes is regulated in vivo by transport of substrates or products across a membrane. A defect in the transport process will only be seen in intact organelles and not a crude tissue homogenate, where the barrier between enzyme and substrate is destroyed. The distinction between glycogen storage disease Ia (GSD Ia von Gierke), in which the enzyme glucose 6-phosphatase is defective and GSD Ib and Ic, in which transport proteins are defective, could be made by assaying the glucose 6-phosphatase in homogenates and microsomes of fetal liver.[26] The activity of a non-specific phosphatase also has to be taken into account in diagnosing these disorders.

In Zellweger's syndrome there is an absence or deficiency of peroxisomes and the marker peroxisomal enzyme, catalase, is localised in the cytosol. This observation can be exploited in several ways for prenatal diagnosis of Zellweger's syndrome by establishing whether (i) catalase is sedimented with organelles after centrifugation of amniocytes or remains in the cytosolic fraction,[36] (ii) the latency of catalase in digitonin-permeabilised, cultured chorionic villi fibroblasts is the same as that for a cytosolic enzyme or for a peroxisomal enzyme,[37] and (iii) peroxisomes can be visualised directly in intact chorionic villi by a cytochemical stain for catalase.[38] These techniques also indicate whether there is a general defect of peroxisomes or a defect in a specific peroxisomal component. Visual discrimination between normal villi and villi from a fetus with Tay-Sachs disease has also been shown by fluorescent cytochemical technique for hexosaminidase A.[33] These approaches could be used to investigate other organelle-specific enzyme dysfunctions.

IMMUNODETECTION

The specificity of antibodies can be used in two ways to investigate enzymic defects. The amount of a particular enzyme activity can be measured selectively by precipitation with monospecific antiserum in the presence of other enzymes acting on the same substrate. Diagnosis of Pompe's disease (glycogenosis II) has been made in chorionic villi by measuring the true acid maltase with antibodies in the presence of other α-glucosidases.[39] Alternatively the presence or absence of an enzyme protein as opposed to its activity can be monitored by immunoblotting (western) or immunocytochemistry. Two enzymes involved in peroxisomal β-oxidation were shown by immunoblotting to be absent from chorionic villi from fetuses with Zellweger's syndrome.[40] A deficiency of immunoreactive alanine:glyoxylate aminotransferase protein was demonstrated by immunoblotting in extracts and subcellular fractions of liver from a fetus with primary hyperoxaluria type I and in peroxisomes directly by protein A-gold immunoelectronmicroscopy.[27] The ultrastructural technique can detect disorders due to the incorrect subcellular localisation of an enzyme, which could not be detected by immunoblotting or assay of a total tissue extract. All immunochemical techniques depend crucially on having monospecific or monoclonal antibodies.

PRECURSOR UTILISATION

The activity of an enzyme may be measured indirectly by the utilisation or incorporation of a radioactively labelled precursor in living chorionic villi or cultured amniotic cells. This procedure has been useful for the diagnosis of several organic acidurias, aminoacidopathies, and defects of nucleic acid metabolism. The incorporation of the precursor, 1 $^{-14}$C citrulline, for the measurement of arginosuccinate lyase in argininosuccinicaciduria is related to incorporation of ^{3}H leucine into protein.[41] For other defects the release of ^{14}C carbon dioxide from the precursor, for example, 1 $^{-14}$C octanoate, for medium chain acyl coenzyme A dehydrogenase is related to protein.[42] However discrepancies have been observed between enzyme activities measured directly and metabolically and confirmation is often required by the identification of accumulating metabolite in the cells or amniotic fluid.

METABOLITE ACCUMULATION

Advances in high performance liquid chromatography, gas chromatography combined with mass spectrometry, electrophoresis, and high performance thin layer chromatography have enhanced the identification and quantitative measurement of specific metabolites in body fluids and cell extracts. Stable isotope analysis with gas chromatography-mass spectrometry detection is a sensitive and selective method, very suitable for prenatal diagnosis. The identification by various methods of a specific metabolite or group of compounds in amniotic fluid obtained at 16–20 weeks has been used extensively to diagnose organic acidaemias,[43] aminoacidopathies,[44] and mucopolysaccharidoses.[45] Most of these disorders can now be diagnosed by direct assay of the relevant enzyme in chorionic villi but metabolite identification in amniotic fluid is often necessary when the first trimester diagnosis is based on uptake of labelled precursor by intact chorionic villi.

RECOMBINANT DNA TECHNIQUES

Much of the pioneering work on the application of recombinant DNA technology to prenatal diagnosis was carried out on the haemoglobinopathies and thalassaemias. The techniques developed in these studies are now being applied to enzyme defects as the genes encoding the affected enzymes are cloned.[46 47] Currently the genes for about 40 enzymes involved in metabolic disorders have been cloned,[48] but only a few of those clones have been used for prenatal diagnosis. Cloned or chemically synthesised DNA sequences encoding all or part of an enzyme gene, gene specific probes, are used to detect or track mutations in genomic DNA associated with the enzymic defect. Differences between the normal and mutant DNA are manifested as different patterns of restriction fragments of DNA in Southern blots analysed with a radioactively labelled gene specific probe. The patterns are different because a mutation in the gene or in a sequence closely linked to the gene has created or obliterated a site recognised by a restriction endonuclease. The theory behind this approach and the methodology are described in detail elsewhere.[46–49]

The essential requirements for prenatal diagnosis of enzymic defects by DNA analysis are careful family studies in cooperation with a clinical geneticist, the probes, DNA from a fetal sample, and a laboratory experienced in the use and interpretation of these techniques. A minimum of 10 μg of DNA is required for analysis. This can be extracted from chorionic villi ($\approx$10 μg of DNA/10 mg wet weight) or cultured cells from the chorionic villi or amniotic fluid ($\approx$6 μg/10^6 cells). Gene specific probes can be used to detect gross abnormalities in genes, such as deletions, insertions, and rearrangements, or, point mutations due to changes in one or a small number of nucleotides.

Detection of gross alterations in a gene

Gross abnormalities are the simplest to detect using probes. They can be useful in correlating a section of a gene with a particular function of the enzyme and perhaps consequent phenotypes. Deletions are uncommon in single copy genes but several have been found in the genes encoding enzymes responsible for genetic disorders[48] and could be used diagnostically within families. A deletion on the short arm of chromosome X was shown to be responsible for a deficiency of glycerol kinase in an aborted fetus.[50]

Detection of a disease-causing mutation

A single base change can affect the transcription and translation of a gene or produce an enzyme protein with a single amino acid change that affects the function of the enzyme. If this base change creates or destroys a restriction endonuclease site, it might be possible to detect it directly by Southern blotting with a gene specific probe. Fewer than about 10% of point mutations can be detected in this way, however, because of the specific structure of restriction endonuclease sites. A mutation in the α-fucosidase gene that obliterates an EcoRI restriction site appears to be the underlying molecular defect in fucosidosis in one group of patients.[51] It is being used to monitor pregnancies at risk for fucosidosis.

Allele-specific oligonucleotides

An alternative method of detecting point mutations can be used if the mutation is known precisely. Two oligonucleotides of about 20 bases are synthesised: one exactly complementary to the normal gene sequence and the other to the mutant gene sequence. By choosing hybridisation conditions where only the 'normal' oligonucleotide will bind to the normal gene sequence and only the 'mutant' oligonucleotide to the mutant gene sequence—that is, complete complementarity is required—it is possible to detect the presence of the two alleles specifically. Allele specific oligonucleotides will be very useful for prenatal diagnosis within families where the mutation has been established. A four base pair insertion in exon 11 of the α-chain of β-hexosaminidase, which has been shown to be the major defect (70% of carriers) in Ashkenazi Jews with Tay-Sachs disease can be detected using two oligonucleotide probes corresponding to the normal and mutant sequences.[52] By constructing a panel of allele specific oligonucleotides for known mutations in an enzyme gene, new cases of known defects and new but unknown mutations could be recognised prenatally. Analysis with oligonucleotide probes can be carried out with 1 μg of DNA, theoretically eliminating the need to culture amniocytes.

A recent development that will be particularly useful for prenatal diagnosis because the amount of DNA is very limited and speed is important, is amplification of a specific DNA sequence by the polymerase chain reaction.[53] A 10^6-fold amplification can be achieved in a few hours using automated equipment. The combination of allele specific probes and polymerase chain reaction of chorionic villi DNA offers a fast, specific, and early diagnosis of known enzymic mutations.

Tracking mutations by linkage to a restriction fragment length polymorphism

It is also possible to detect or track a mutant gene without knowing the nature of the molecular defect by linkage analysis based on DNA polymorphisms. Normal variation occurs frequently in the human genome. This can generate alleles, which give rise to different lengths of DNA when digested with a restriction endonuclease and analysed by Southern blotting. Such restriction fragment length polymorphisms can be used to track a mutant gene if a polymorphism is in the chromosome with the mutant gene but not in the normal gene or vice versa. As these polymorphisms are common there are usually several within a gene or very close to it—that is, they are tightly linked to it and will be detected by Southern blot analysis using the gene specific probe. It should be emphasised that the polymorphism is not the mutation causing the enzyme defect but it can be used to track or identify the mutant gene. Several enzymic defects have been diagnosed prenatally using restriction fragment length polymorphisms in DNA from chorionic villi or cultured aminocytes (see appendix). DNA from the index case, parents and some other members of the family must be analysed to establish the phase between a polymorphism and the mutant gene—that is, whether the polymorphism is on the normal or mutant chromosome. The parents must be heterozygous and the true parents.

Ten independent restriction fragment length polymorphisms have been found linked to the phenylalanine hydroxylase locus. Appropriate haplotypes, that is, a combination of polymorphism and mutant gene, have been found for most families with a phenylketonuria mutation, permitting detection of carriers and diagnosis of affected individuals. It has been found that most cases can be diagnosed prenatally using three restriction enzymes (polymorphisms).[54] Prenatal diagnosis by DNA analysis of phenylketonuria and the X linked ornithine carbamoyl transferase deficiency[55] is very valuable because these enzymes are only expressed in tissues not easily accessible to fetal sampling.

For many common genetic disorders the underlying gene defect is unknown but DNA polymorphisms can still be used to track the mutant gene if tight linkage can be shown between the disorder and a polymorphism detected with an adjacent marker DNA probe. This approach can be used for an enzyme defect where the enzyme gene has not been cloned but it is known to be closely linked to well defined markers that have been cloned. The enzyme, steroid 21-hydroxylase, a deficiency of which is the most common cause of congenital adrenal hyperplasia, is situated within the major histocompatibility complex. This disorder can now be diagnosed in the first trimester using HLA-DNA probes and linkage analysis together with 17-hydroxyprogesterone determination.[56] Previously prenatal diagnosis was based on HLA serotyping of cultured amniocytes and measurement of 17-hydroxyprogesterone in amniotic fluid.

Many of the new developments in DNA diagnostics will be applied to prenatal diagnosis. They include the more widespread use of non-radioactive probes exploiting fluorescence, chemiluminescence, or the high affinity between biotin and streptavidin or avidin linked to an indicator enzyme, full automation of DNA extraction, and analysis and detection of mutations in solution without immobilisation of nucleic acid.[57]

Future developments

The greater availability and utilisation of chorionic villus sampling will increase the number of enzyme defects that are diagnosed in the first trimester. This will offer families the advantages of early diagnosis and more safe and reliable termination of a pregnancy. DNA analysis will make possible the early diagnosis of many of those disorders previously only able to be diagnosed in the second trimester. The possibility of diagnosis of genetic errors, including enzymopathies, in preimplantation embryos is being investigated.[58] [59] Diagnosis of Lesch-Nyhan syndrome by enzyme assays and the sexing of embryos using Y specific DNA probes are technically feasible. The low rate of implantation and other embryological factors present considerable problems, however, apart from ethical considerations. Another situation with ethical implications and technical complications is prenatal diagnosis in multiple pregnancies and the possibility of selective termination. This has been carried out after amniocentesis and chorionic villus sampling.[60] Recently a twin pregnancy at risk for sialic acid storage disease was investigated in our laboratory. On the basis of a direct biochemical assay for sialic acid and morphological examination of chorionic villi, selective fetocide was carried out.[61]

The list of enzyme defects that can be diagnosed prenatally grows inexorably.[62] Most of these are included in the following appendix.

Appendix

Enzyme defects that have been diagnosed prenatally

Within each section the names of the diseases are in alphabetical order. Unless otherwise indicated diagnosis is based on measurement of enzyme activity or metabolites in chorionic villi, amniotic fluid, or cultured amniotic cells. (*Fetal blood or liver; †newly recognised enzyme defects for which methodology is available; ‡DNA analysis possible.)

Disorder	McKusick No	Defect
AMINO ACID AND ORGANIC ACID METABOLISM		
Canavan†	27190	Aspartoacylase
Cystinosis	21980	Cystine transport protein
Glutaric aciduria type I	23167	Glutaryl-CoA-dehydrogenase
Glutaric aciduria type IIA	30595	Multiple acyl-CoA dehydrogenases
Glutaric aciduria type IIB	23168	Multiple acyl-CoA dehydrogenases
Gyrate atrophy‡	25887	Ornithine aminotransferase
Homocystinuria	23620	Cystathionine β-synthase
3-Hydroxy-3-methylglutaryl CoA lyase deficiency	24645	
Maple syrup urine	24860	Branched chain ketoacid decarboxylase
Methyl malonic aciduria:		
Vitamin B12 non-responsive	25100	Methylmalonyl-CoA mutase
Vitamin B12 responsive	25110	Adenosylcobalamin synthesis
Mevalonic aciduria	25117	Mevalonate kinase
Methylene tetrahydrofolate reductase deficiency	23625	
Multiple carboxylase deficiency	25327	Holocarboxylase synthase
Oculocutaneous albinism	20310	Tyrosinase
Phenylketonuria (PKU I)‡	26160	Phenylalanine hydroxylase
Phenylketonuria (PKU II)‡	26163	Dihydropteridine reductase
Phenylketonuria (PKU III)*	26164	Tetrahydrobiopterin synthesis, 6-pyruvoyl tetrahydropterin synthase

Propionic acidaemia	23200	Propionic CoA carboxylase
Pyroglutamic aciduria	26613	Glutathione synthase
Tyrosinaemia I	27670	Fumarylacetoacetate hydrolase
UREA CYCLE ENZYMES		
Argininosuccinic aciduria	20790	Argininosuccinate lyase
Carbamoyl phosphate synthase deficiency	23730	
Citrullinaemia	21570	Argininosuccinate synthase
Ornithine carbamoyltransferase deficiency*‡	31125	
CARBOHYDRATE METABOLISM		
Fructose-1:6 diphosphatase deficiency	22970	
Galactosaemia	23040	Galactose-1-phosphate uridyltransferase
Galactosaemia	23020	Galactokinase
Glycerol kinase deficiency‡	30703	
Glycogenoses		
Type Ia von Gierke*	23220	Glucose 6-phosphatase
Type II Pompe	23230	Acid α-glucosidase (acid maltase)
Type III Cori	23240	Amylo-1,6-glucosidase (debrancher enzyme)
Type IV	23250	α-1, 4-Glucan: α-1,4 glucan 6-glucosyltransferase (brancher enzyme)
Pyruvate carboxylase deficiency	26615	
STEROID METABOLISM		
X linked ichthyosis‡	30810	Steroid sulphatase
Congenital adrenal hyperplasiaIII‡	20191	Steroid 21-hydroxylase
Congenital adrenal hyperplasia IV	20201	Steroid 11-β-hydroxylase
PEROXISOMAL AND MITOCHONDRIAL DISORDERS		
Zellweger	21410	Multiple enzyme deficiencies
Infantile Refsum	26651	
Neonatal adrenoleucodystrophy	20237	
Rhizomelic chondrodysplasia punctata	21510	Acyl-CoA: dihydroxyacetone phosphate acyltransferase and alkyl dihydroxyacetone phosphate synthase
X linked adrenoleucodystrophy‡	30010	Very long chain acyl-CoA synthase
Other defects in peroxisomal β-oxidation including Pseudo-Zellweger	26151	3-Oxoacyl-CoA thiolase
Primary hyperoxaluria type I*	25990	Alanine: glyoxylate aminotransferase
Refsum (classical)	26650	Phytanic acid α-hydroxylase
Cytochrome C oxidase deficiency	22011	
Medium chain acyl-CoA dehydrogenase deficiency	20145	
LYSOSOMAL STORAGE DISEASES		
Sphingolipidoses and neutral lipidoses		
Fabry‡	30150	α-Galactosidase A
Farber	22800	Ceramidase
Gaucher:		
Type I	23080	Glucocerebrosidase
Type II	23090	
Type III	23100	
G_{M1} Gangliosidosis:		
Type I (Infantile)	23050	β-Galactosidase
Type II (Juvenile)	23060	
G_{M2} Gangliosidosis:		
Tay-Sachs	27280	β-Hexosaminidase A
Sandhoff	26880	β-Hexosaminidase A and B
AB variant	27275	G_{M2}-ganglioside activator protein
Krabbe	24520	Galactosylceramide β-galactosidase
Metachromatic leucodystrophy	25010	Arylsulphatase A or activator protein
Mucolipidosis type II (I-cell)	25250	N-Acetylglucosamine 1-phosphotransferase
Mucolipidosis type III (pseudo-Hurler polydystrophy)	25260	
Mucolipidosis IV	25265	Ganglioside neuraminidase
Sulphatidosis	27220	Multiple sulphatases

Niemann-Pick:		
Type A, B	25720	Sphingomyelinase
Type C	25722	Cholesterol esterification
Cholesterol ester storage	21500	Acid lipase
Wolman	27800	
Glycoprotein metabolism		
Aspartylglucosaminuria	20840	N-aspartyl-β glucosaminidase
Fucosidosis	23000	α-Fucosidase
Galactosialidosis	25654	β-galactosidase and α-neuraminidase protective protein
α-Mannosidosis	24850	α-Mannosidase
β-Mannosidosis†	24851	β-Mannosidase
Mucolipidosis I (Sialidosis)	25655	α-Neuraminidase
α-N-acetylgalactosaminidase deficiency†	10417	
Salla	26874	Lysosomal transport of sialic acid
Sialiac acid storage	26992	
Mucopolysaccharidoses		
Hurler/Scheie (MPS I)	25280	α-Iduronidase
Hunter (MPS II)	30990	Iduronate sulphate sulphatase
Sanfilippo:		
A (MPS IIIA)	25290	Heparan sulphamidase
B (MPS IIIB)	25292	N-acetyl α-glucosaminidase
C (MPS IIIC)	25293	Acetyl-CoA: α-glucosaminide N-acetyltransferase
D (MPS IIID)	25294	N-acetylglucosamine-6-sulphate sulphatase
Morquio		
A (MPS IVA)	25300	N-acetylgalactosamine 6-sulphate sulphatase
B (MPS IVB)	25301	β-Galactosidase
Maroteaux-Lamy (MPS VI)	25320	Arylsulphatase B (N-acetylgalactosamine 4-sulphate sulphatase)
Sly (MPS VII)	25322	β-Glucuronidase
PHOSPHATASES		
Acid phosphatase deficiency	20095	Lysosomal phosphatase
Hypophosphatasia	24150	Bone/liver alkaline phosphatase
NUCLEIC ACID METABOLISM		
Combined immunodeficiency	10270	Adenosine deaminase
Lesch-Nyhan‡	30800	Hypoxanthine guanine phosphoribosyltransferase
T cell immunodeficiency	16405	Purine nucleoside phosphorylase
Xanthine/sulphite oxidase	25215	Molybdenum cofactor
Xeroderma pigmentosum	27872	DNA excision and repair
HAEMATOLOGICAL DISORDERS		
Acute intermittent porphyria	17600	Porphobilinogen deaminase
Congenital haemolytic anaemia	17240	Glucose phosphate isomerase
Congenital erythropoietic porphyria	26370	Uroporphyrinogen III synthase
Methaemoglobinaemia type II	25080	NADH-diaphorase (NADH cytochrome B5 reductase)
Triose phosphate isomerase deficiency	19045	

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PERINATAL LESSONS FROM THE PAST

Dr von Reuss on continuous positive airway pressure in 1914

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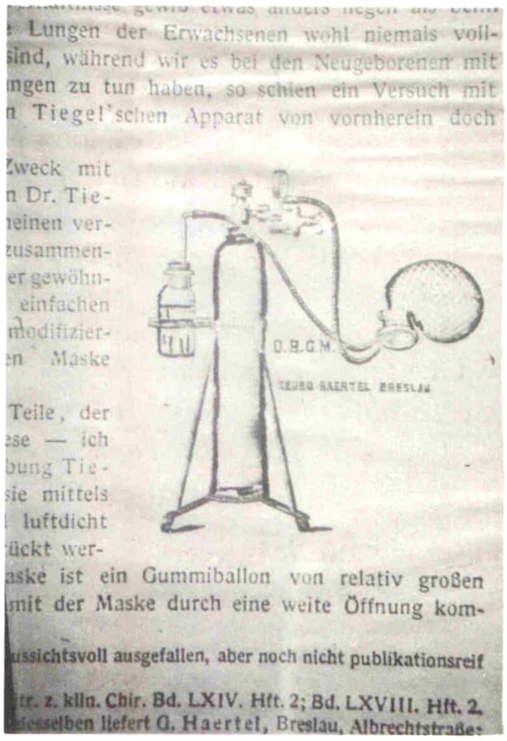
The classic text *Diseases of the Newborn* by August Ritter von Reuss was first published in Germany in 1914, with an English translation in 1921.[1] Von Reuss, who incidentally was the first to describe galactosaemia in 1908, had been appointed director of the Department for the Newborn at the First University Women's Clinic in Vienna in 1911 and therefore also has right to the title of being the first 'neonatal paediatrician'. Interestingly, in the foreword of his book he writes: 'the newborn period . . . includes that period of time in which the vital processes are still under the influence of those violent changes involved by the abrupt transition from intra-uterine to extra-uterine life . . . We find ourselves here on the borderline between paediatrists and obstetricians . . . To the question, On whom does the care of the newborn lie? there is only one answer: On the specialist. Whether he is an obstetrician or a paediatrist is then of no consequence'.

The *Diseases of the Newborn* is amazingly complete and modern in its approach to the physiology and pathology of the newborn as the following review of the use of continuous airway pressure in resuscitation of the newborn reveals, a technique that was only rediscovered in 1971 for the treatment of respiratory distress syndrome.[2]



Lungen der Erwachsenen wohl niemals voll-
sind, während wir es bei den Neugeborenen mit
ngen zu tun haben, so schien ein Versuch mit
n Tiegel'schen Apparat von vornherein doch

Zweck mit
n Dr. Tie-
neinen ver-
zusammen-
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Von Tiegel's 'over-pressure apparatus' (1911).

'Hoerder[3] recommends a modification of the so-called "over-pressure apparatus" of Brat and Schneider. The method makes it possible with the right pressure to keep the lung expanded during the phase of expiration, without obstructing the retraction required for ventilation of the lung. The over-pressure is produced by impeding the breathing by the application of an expiration valve. This method is carried out by a tracheal catheter, marked so that it can be introduced to a little above the bifurcation. First the mucus is aspirated into a small glass bowl and then oxygen insufflated. The pressure is regulated by a water valve. The pumping of oxygen (about thirty to forty times a minute) is only to be stopped when the child has obtained a healthy colour, and the catheter only removed when the child breathes regularly and spontaneously. Artificial respiration by means of this apparatus is supposed to act better if the child's breathing has completely stopped.

'Englemann's[4] method of insufflation of oxygen at a pressure aims at expansion of the infantile lung without catheterization of the trachea. Von Tiegel's apparatus (figure) consists of an ordinary oxygen cylinder with a simple water valve and a suitable face mask. This is so constructed that it can be applied to the face by means of a hermetically closing rubber ring. A relatively large rubber ball is screwed to the top of the mask, and communicates with it through a wide opening. A piece of tube is attached to the interior of the mask, and through this the stream of gas is conducted from the cylinder to the ball. From here it is partly inspired, and is partly mixed with the expired air, together with which it passes through an outlet in the lower part of the mask into a tube, and from thence into a water pressure regulator. This consists of a simple metal tube that is placed in a receptable filled with water and provided with a centimetre scale. According to the depth of immersion of the metal tube the pressure can be varied in the whole system to a desired point. Quite a small pressure (2 to 3 cm. water) is sufficient to start respiration.'

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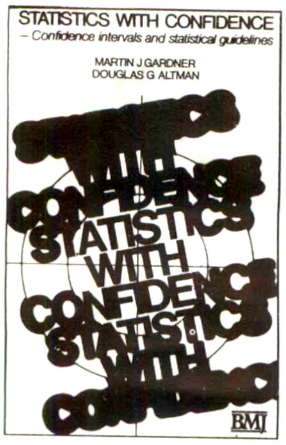
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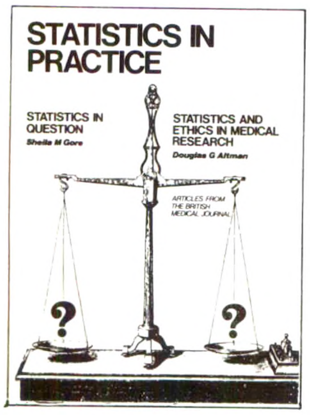
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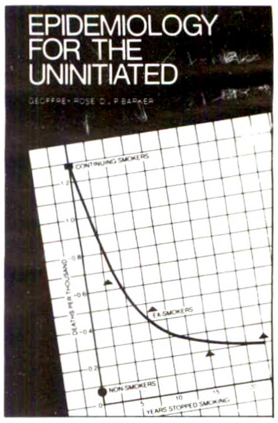

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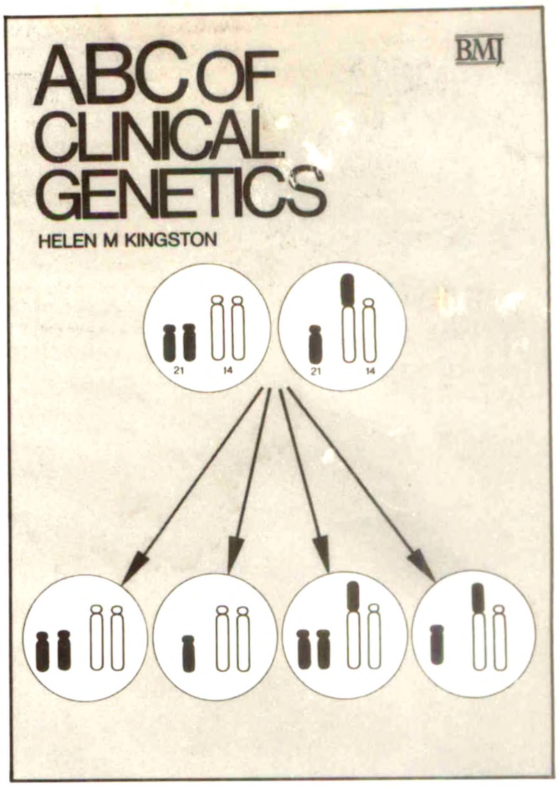

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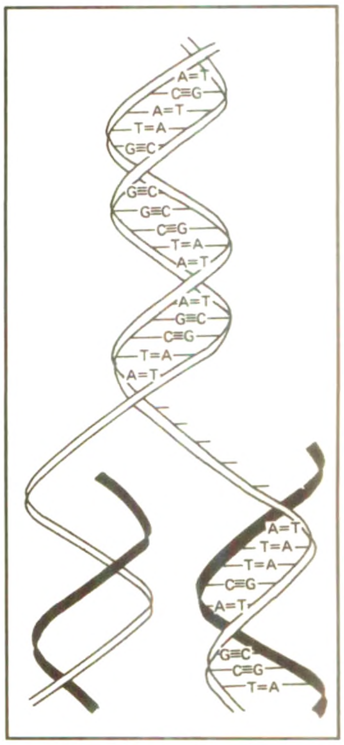

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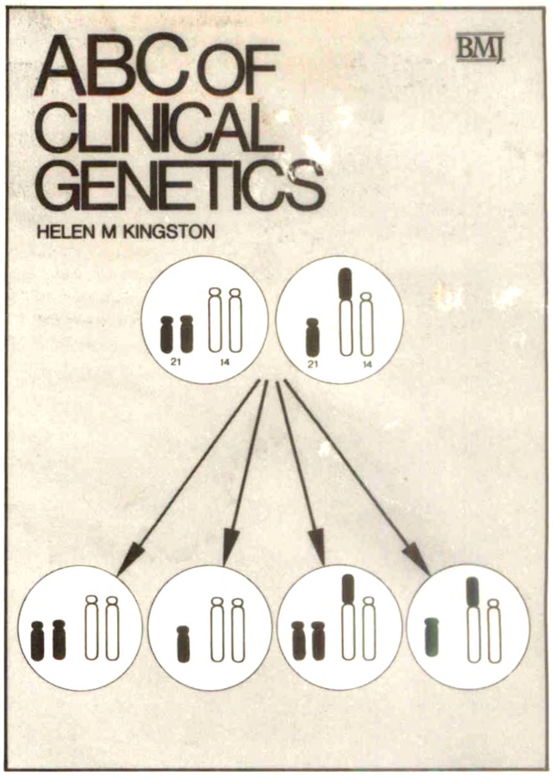

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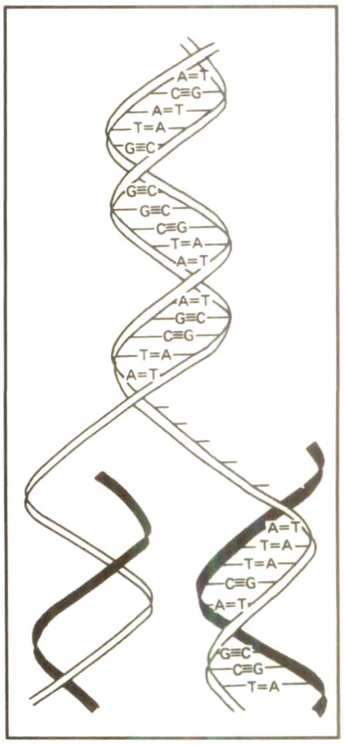

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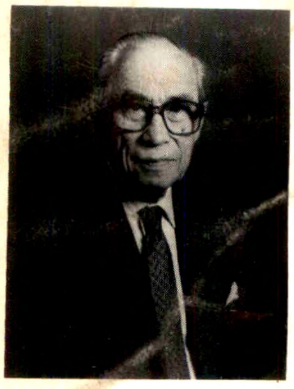

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Annotations

Transcutaneous carbon dioxide monitoring

The skin of a preterm baby has a thin epidermis and is poorly keratinised, which facilitates the measurement of oxygen, carbon dioxide, and bilirubin, and provides an absorptive surface for drugs and oxygen.[1 2] The idea of transcutaneous monitoring developed after the permeability of skin was described in 1851,[3] and was first used successfully in 1972.[4] Miniature devices for measuring blood gas tension have now become usefully established in many neonatal units. The continuous availability of measurements of oxygen tension has alerted those who work in nurseries to the short term fluctuations that can occur during apparently innocuous procedures such as feeding, noise, and nappy changes.[5] Maintenance of a constant carbon dioxide tension is equally as important as a stable oxygen tension in babies with respiratory failure: why, then, has transcutaneous carbon dioxide monitoring 'never come out of the oven'? (P Rolfe, 3rd International Congress of Fetal and Neonatal Physiological Measurements, Sweden, 1988).

Need for carbon dioxide monitoring

Frequent analyses of both arterial oxygen and carbon dioxide are essential to assess the adequacy of support in babies with respiratory failure. The usual diagnosis will be that of the respiratory distress syndrome, but the group includes babies ventilated for meconium aspiration, pulmonary hypoplasia, pneumonia, myopathy, and birth asphyxia. Hypercarbia is a potent vasodilator and has been implicated in the aetiology of periventricular haemorrhage,[6] and hypocarbia with its vasoconstrictor effect may reduce cerebral blood flow and contribute to ischaemic lesions of the brain.[7] Early detection of a rising carbon dioxide tension sometimes provides warning of blockage of an endotracheal tube at a stage when suction is sufficient to clear the secretions, obviating the need for the endotracheal tube to be changed. A pneumothorax can be diagnosed before the development of tension results in serious cardiovascular consequences.[8] After extubation knowledge of accumulating carbon dioxide can be particularly helpful in identifying babies who are making inadequate respiratory effort before they develop acidosis and bradycardia, and the same is true after operation and during transport.

During the treatment of persistent pulmonary hypertension it may be necessary to achieve a carbon dioxide tension as low as 2 kPa before reducing the pulmonary arterial pressure.[9] Hyperventilation and maintenance of hypocarbia is also indicated in some cases of birth asphyxia.[10] There seems little doubt, therefore, that continuous monitoring of carbon dioxide tension can assist clinical care.

Technique of measurement

The most commonly used type of electrode is based on the pH determination of a thin film of electrolyte separated from the skin by a hydrophobic membrane permeable to carbon dioxide but not to hydrogen ions. When carbon dioxide diffuses through the membrane there is a change in the pH of the electrolyte, as carbon dioxide reacts with water to form hydrogen and bicarbonate ions. Determination of the pH of the solution thus gives an indirect measure of carbon dioxide, the potential difference between the pH electrode and a metal reference electrode being proportional to the logarithm of the partial pressure of carbon dioxide.[11] This method of measurement does not use a polarographic electrode and so does not consume carbon dioxide. During transcutaneous measurement of oxygen using a polarographic electrode a cathode and anode have a polarising voltage passed through them, which reduces oxygen and results in the formation of hydroxyl ions and the production of a current proportional to the amount of oxygen present.[12]

By combining the reference electrodes and providing a cathode for oxygen and a glass pH electrode, a combined sensor can be made.[13] For each oxygen molecule consumed in this type of electrode four electrons are produced and hence four hydroxyl pairs. These will react with any carbon dioxide present to form bicarbonate, causing the partial pressure of carbon dioxide in the solution to reduce over time: Severinghaus has described an elegant solution to the design of combined oxygen/carbon dioxide sensors in which the hydroxyl produced at the cathode is consumed at the anode.[14]

Accuracy of measurement

For transcutaneous measurements to reflect arterial blood gas tensions it is necessary to warm the skin, 'arterialising' the capillaries. In combined electrodes the operating temperature has to be 44°C to promote skin hyperperfusion and to obtain a correlation between arterial and skin oxygen tension. Heating the electrode also improves the response time. Carbon dioxide is more readily diffusable than oxygen, and carbon dioxide electrodes can be operated satisfactorily at lower temperatures than oxygen sensors. A better correlation with arterial blood gas tension is usually obtained at higher operating temperatures,[15] although Wimberley *et al* found an increasing difference with increasing operating temperature.[16]

In spite of arterialisation of the capillaries, the transcutaneous carbon dioxide measurement is always greater than the corresponding arterial measurement; the overreading results partly from local tissue production of carbon dioxide (amounting to about 0·5 kPa)[17] and partly from the heating coefficient of blood. The readings are approximately 1·27 times or 27% higher. A correction factor of 0·77 can be applied to the value obtained from a transcutaneous monitor to estimate the arterial partial pressure.

A transcutaneous carbon dioxide electrode needs calibrating against a known concentration of carbon dioxide every time it is removed from a baby, which needs to be at least every four hours to protect the skin and is often more frequent. After calibration a period of stabilisation of about 10 minutes is required before readings become stable. The time constant (63% response time) for changes in carbon dioxide tension after this is about 60 seconds.[13] [18] Several careful studies have examined the accuracy of transcutaneous monitors in a clinical setting. Martin *et al* studied 60 newborn infants with a mean birthweight 2200 g.[19] Forty eight had respiratory distress syndrome, and none was hypotensive. Transcutaneous carbon dioxide tension was accurate over the hypocarbic range, but overestimated by a mean (SD) of 0·66 (0·53) kPa in the normal range and by 1·2 (0·8) kPa in the hypercarbic range. The values deviated from the line of identity even after the type of correction described above, with a slope that increased as the amount of carbon dioxide increased. The authors thought that there may have been an imbalance between carbon dioxide production and removal in hypercarbia, perhaps as a result of local shunting. A Danish study of 64 newborns with respiratory distress confirmed the overestimation shown by others, with a correction factor of 0·7 resulting in correct grouping of 82% of the values.[19] Sensitivity and specificity were between 72% and 88% with a coefficient of variation of 5·3%. Overall, the correlation between arterial and transcutaneous measurements was good, with r=0·9 to 0·95 at different electrode temperatures. Large drifts were experienced, however, with 543 check calibrations showing a mean drift of +2·6%, SD 6%, and range 33%. Similar problems were experienced by Kost *et al*,[20] who found a drift larger than 0·4 kPa/hour in 10% of 500 monitoring sessions, and at University College Hospital,[13] where the calibration against 10% carbon dioxide drifted down at 0·3 kPa/hour. Lee *et al* evaluated two combined sensors in older children and found them to be generally accurate, although the oxygen electrode had a reduced performance when compared with a single sensor.[21] Drift was small for the carbon dioxide electrode, at −0·2 kPa in four hours. The reason for the drift is not known, but because it is larger in vivo than when an electrode is left in calibration gas, one explanation has been that it is due to pressure sensitivity of the electrode.

Limitations of the method

In the appropriate setting, surface carbon dioxide tension can be used to predict arterial carbon dioxide tension with a reliability similar to that of transcutaneous oxygen monitoring. The difficulty of remembering to apply a correction factor can easily be overcome by adjusting the sensor to underestimate the calibration gas—for example, to read 7 kPa in 10% carbon dioxide. The method is free from serious side effects, although skin craters have been described.[22] The cost of commercial equipment ranges from £2000 to £6000, the electrodes are fragile and expensive, and the systems require special calibration gas. The result is not reliable in shocked babies,[23] and although there have been no adverse effects shown from hypothermia, acidosis, sclerema, or anaemia, all these factors are known to affect transcutaneous oxygen estimation and the presence of any of them would make most clinicians unwilling to rely on transcutaneous monitoring.[24] increasing thickness of the epidermal layer with increasing postnatal age makes the method inaccurate in bronchopulmonary dysplasia.[25]

Conclusion

In spite of the attractions of continuous carbon dioxide monitoring, and the availability of combined oxygen and carbon dioxide sensors, the method has yet to become established in neonatal intensive care. Used carefully there is no doubt that it is capable of providing an accurate result for a clinically important variable. The limitations are not only cost, but also the fragility of the equipment, and the need for repeated calibration against a gas other than air (followed by the considerable time taken for stabilisation). Babies with variable carbon dioxide tensions are often those who are hypotensive, and who require repeated investigations necessitating resiting of electrodes. This results in readings that are unreliable and often unavailable at a crucial moment, which has perhaps led to disillusion. To continue the analogy from the introduction, perhaps the next decade will enable continuous non-invasive carbon dioxide monitoring to be 'fully baked'.

J M RENNIE

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Treatment of sepsis with IgG in very low birthweight infants

Despite the continued development of new antibiotics with increased ranges of activity and spiralling prices, sepsis—either acquired from the mother intrapartum or acquired nosocomially from invasive procedures—remains a serious problem in preterm infants. All neonatologists must have witnessed infants dying of septicaemia despite treatment with an antibiotic to which the organism was sensitive in vitro. The problem is largely one of host defence. Infants born before 32 weeks' gestation are seriously immunodeficient with cord blood concentrations of IgG being less than half those found in babies born at full term. Concentrations of IgA and IgM are negligible in cord blood. In addition, very preterm infants have reduced complement factors, opsonic activity, polymorphonuclear chemotaxis, and are liable to exhaust their neutrophil storage pools.[1]

As IgG is acquired across the placenta, and the infant produces virtually none of its own for months, serum IgG concentrations decline from birth for six to eight weeks reaching 1–2 g/l between 2 and 4 months of age; these low concentrations must be of functional importances. Opsonisation of important capsulated perinatal pathogens such as group B streptococi, *Escherichia coli* and *Staphylococcus epidermidis* is facilitated by specific antibody.[2] Release of marrow neutrophils is accelerated by type specific antibody,[3] which also speeds up migration of neutrophils into the infected tissue.[4]

Accepting that infants born at less than 32 weeks' gestation have clinically important immmunoglobulin deficiency, can they be effectively and safely treated with replacements? What preparations are suitable? What size of dose and number of doses are required? What is the evidence of efficacy?

Immunoglobulin preparations

Early preparations of human gammaglobulin were not suitable for intravenous injection because of the presence of impurities and aggregated immunoglobulin, which gave rise to complement activation and anaphylaxis. The early preparations could safely be given intramuscularly but insufficient volume could be injected to raise the serum concentrations of IgG to those of a full term infant. Highly purified human immunoglobulin suitable for intravenous use has been available for the last decade. Sandoglobulin (Sandoz), is one of the most widely used in Europe. It is a Swiss Red Cross pooled polyvalent IgG concentrate produced by alcohol cryoprecipitation and mild acidification at pH 4 from plasma known to be negative for hepatitis B antigens and antibodies to HIV. Gammagard (Baxter Health Care), Intraglobin (Biotest Pharma), and Venogamma Polivalente (Ismunit) are also IgG concentrates made by ethanol fractionation of plasma. An IgM enriched immunoglobulin has recently been developed (Pentaglobin, Biotest Pharma). A dose of 250 mg of protein in 5 ml contains 190 mg IgG, 30 mg of IgM, and 30 mg of IgA.

Dosage

Chirico *et al* showed that 500 mg/kg Sandoglobulin given to preterm infants under 1500 g raised the mean serum IgG concentration to over 13 g/l, with a serum half life of 260 hours.[5] Antibody titres to *E coli* rose to a concentration that is normally protective, and with a serum half life of 112 hours. Antibody titres to group B streptococcus rose to normally protective concentrations with a serum half life of 82 hours, and antibody to cytomegalovirus also rose appreciably with a serum half life of 112 hours. Noya *et al* studied pharmacokinetics of Gammagard in 20 infants weighing 750–1500 g during the first week.[6] Mean IgG rose from around 5 g/l to over 13 g/l (equal to the concentrations in full term infants or adults) 15 minutes after infusion of 500 mg/kg. Mean IgG had fallen to 7·3 g/l by 7 days, to 6·1 g/l by 14 days, to 5·1 g/l after 21 days, and to 4·3 g/l after 28 days.

Toxic effects

No liver damage or anaphylaxis has been reported in newborns. The theoretical possibility that administration of IgG early in life might suppress later development of endogenous IgG was investigated by Sidiropoulos.[7] He followed up a group of infants treated with Sandoglobulin in the neonatal period and found normal serum concentrations of IgG and antibodies to tetanus toxoid at 4 years of age. Ethanol fractionation of plasma has been shown to inactivate HIV.[8]

Efficacy

IgG replacement may be carried out prophylactically in infants at high risk of sepsis (for example <32 weeks' gestation, <1500 g in weight) or as 'rescue' treatment in infants already ill with sepsis. Four randomised trials of prophlaxis have been published. Haque *et al* randomised 150 preterm infants in Saudi Arabia into three groups of 50; one group received a single infusion of 120 mg/kg of Intraglobin on day 1, a second group received two such infusions, the first on day 1 and the second on day 8, and the third (control) group received no immunoglobulin.[9] Culture positive infections (five septicaemia, two meningitis, and one salmonella gastroenteritis) developed in eight infants (16%) of the control group and in four (4%) in the two immunoglobulin treated groups (four with septicaemia). This difference in incidence of sepsis is significant. Although 120 mg/kg is a smaller dose than that used by most other investigators, Haque *et al* found a satisfactory rise in serum IgG.

In another prophylactic trial, 500 mg/kg of Sandoglobulin was given intravenously weekly for one month to infants weighing less than 1500 g in a randomised open trial (83 infants).[5] Only two of the treated infants (5%) developed septicaemia compared with eight of the control group (20%). Of the control group 15% died with infection as the main cause, compared with 2% of the treated group. The same investigators carried out a prophylactic trial in infants over 1500 g who were receiving intensive care and found no difference in outcome.[5]

Stabile *et al* carried out an open randomised prophylactic trial in which they gave 500 mg/kg of Venogamma Polivalente on the first, second, third, seventh, 14th, 21st, and 28th days in 94 preterm infants with a mean gestational age of 31 weeks and a mean birth weight of 1340 g.[10] They found no difference in the incidence of sepsis or mortality between the two treatment groups. The results of this trial are perhaps not directly applicable to very preterm infants in other countries because many of the infants were over 1500 g or 30 weeks, the range of organisms was unusual with not a single case of group B streptococcus or *E coli*, and the immunoglobulin preparation is different from that used in all other neonatal studies and is not available in most countries. Didato *et al* carried out a prophylactic trial of intravenous IgG but with insufficient statistical power (only 37 infants under 1500 g); no significant difference was found.[11]

Sidiropoulos *et al* carried out a randomised open 'rescue' trial in infants ill with suspected sepsis.[12] Sandoglobulin was given to half the preterm infants in a dose of 500 mg/day for a total of six days. Sepsis was confirmed in 22 preterm infants. Four out of nine preterm infants in the control group and one out of 13 preterm infants in the group receiving immunoglobulin died ($p<0·04$).

Haque *et al* carried out a double blind 'rescue' trial.[13] Sixty infants ill with suspected sepsis (later confirmed in 44) were randomised and 250 mg/kg Pentaglobin was given daily to half the infants. Mortality from sepsis was significantly higher, being six out of 30 (20%) in the control group, and one out of 30 (3%) in the immunoglobulin treated group.

Conclusion

The published evidence shows a pronounced, prolonged deficiency of immunoglobulin in very preterm infants, which places them at increased risk of bacterial sepsis. Highly purified and apparently safe intravenous immunoglobulin preparations can restore serum IgG concentrations to those of full term infants or adults. Two out of four randomised trials concluded that prophylaxis could reduce septicaemia in infants weighing <1500 g and two 'rescue' trials have shown improved survival in septic neonates. Large randomised trials are in progress in the United States. Whether neonatologists opt to use IgG prophylactically in the highest risk group (those weighing under 1000 g receiving intensive care) will depend on the background incidence of bacterial sepsis in their own neonatal units. There are other ways of reducing the incidence of sepsis, such as recruiting adequate numbers of qualified nurses, strict handwashing, and avoidance of invasive procedures.

Further research is needed on the minimum effective dosage and the interval between doses, but in units where infection is common in very low birthweight infants it is reasonable (on available data) to treat prophylactically with 500 mg/kg on admission and to repeat treatment every one to two weeks. In the absence of hypovolaemia, IgG should be given slowly over three hours, as the volume of Sandoglobulin is 17 ml/kg with 2·5 mmol of sodium/kg. In the United Kingdom, Sandoglobulin costs £19·50 for 1 g and £46·80 for 3 g. We have found that treating two or three infants out of the same bottle on the same day reduces the cost to acceptable levels. Alternative approaches would be to monitor serum concentrations of IgG weekly in infants weighing under 1500 g and treating infants whose concentrations fell below 3·0 g/l,[14] or to reserve IgG for immediate use in very low birthweight infants who became ill with suspected sepsis. Whichever policy is adopted, IgG will not protect against all types of infection. Fresh frozen plasma is also required to restore complement dependent opsonisation fully. In the future, human monoclonal antibody preparations against the common perinatal pathogens will probably provide more effective treatments in smaller volumes of fluid.[15] The increasing numbers of very preterm infants being treated with dexamethazone for bronchopulmonary dysplasia are likely to have decreased cell mediated immunity as well as immunoglobulin deficiency.

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ORIGINAL ARTICLES

Alfentanil pharmacokinetics in preterm infants

N Marlow, A M Weindling, A Van Peer, J Heykants

Abstract

The pharmacokinetics of alfentanil were studied during the first four days after birth in 22 ventilated preterm infants who were all receiving muscle relaxants. Five minutes after a single dose of 20 µg/kg alfentanil median serum concentration was 66 ng/ml (range: 20–606). The median clearance ws 0·87 ml/kg/min (range: 0·4–9·62) and median elimination half life 321 mins (64–1251). There were wide differences in the manner in which individual infants handled the drug and transient depression of blood pressure and heart rate was observed. These data were used to calculate an infusion dosage. In four infants 20 µg/kg alfentanil given by infusion over 30 minutes followed by 5 µg/kg/hour produced steady state median alfentanil concentrations of 54·5 ng/ml (range: 7–73 ng/ml) with no evidence of drug accumulation.

There is a dichotomy of opinion among paediatricians as to whether newborn infants receiving mechanical ventilation should receive sedative drugs, either to improve the match between the infant's own respiratory effort and that of the ventilator or to relieve their perceived discomfort. In 1984 only 8% of British neonatal units routinely administered sedation to ventilated newborn infants.[1] In a recent postal questionnaire survey involving 32 regional and district newborn intensive care units this figure had risen to 18%, a further 50% using sedation on an occasional basis and 32% using no sedation at all (J Waugh, personal communication, 1988). The use of analgesics has recently been thrown into relief by several recent publications concerning the response of the preterm child to surgical stress.[2,3] These data have been used to suggest that medical intensive care of the newborn should also be accompanied by sedation or analgesia,[3,4] even on a priori grounds without supporting data.[5]

Published data relate to how the term infant handles sedative and analgesic drugs. These data are derived from single dose studies, performed around planned surgical procedures. Fentanyl, for example, has an appreciably prolonged elimination half life and diminished clearance when given to preterm infants.[6] This is accompanied by a wide variation between subjects. Monitoring drug effect and preventing toxicity are thus problematical, especially if muscle relaxants are used. In view of these uncertainties we have studied the pharmacokinetics of a short acting, synthetic opioid, alfentanil, in order to assess the practicability of opiate infusions for ventilated newborn infants.

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Patients and methods

This study was conducted in two phases: first, alfentanil pharmacokinetics were established in preterm infants after a single dose; secondly, these data were used to calculate a dose for infusion and alfentanil concentrations were determined during continuous infusion.

PHASE I—SINGLE DOSE STUDY

A total of 20 µg/kg alfentanil (Rapifen, Janssen Pharmaceutical) was administered by peripheral intravenous infusion over two minutes to 22 subjects, by one investigator (NM). All were preterm infants receiving mechanical ventilation with a clinical diagnosis of hyaline membrane disease. No mother had received alfentanil during the perinatal period. All infants were receiving pancuronium 80 µg/kg at a frequency sufficient to abolish visible movement. All infants had indwelling arterial catheters. Heart rate, mean blood pressure, and transcutaneous oxygen tension were recorded continuously and noted before and at one minute intervals after the injection, for up to 15 minutes. Informed parental consent was obtained for all infants.

Arterial blood (0·4 ml) was withdrawn at the following time points: 0 (control), 5, 15, 60, 90, 180, 300, and 420 minutes after injection. Blood gases (0·2 ml blood) were measured before the injection and five minutes after. Thereafter measurements were taken as clinically indicated, at least every four hours. The maximum number of samples taken was eight, three being omitted where the birth weight was 1000 g or less. Samples were spun, separated, and deep frozen until analysis. Serum concentrations of alfentanil were determined in 0·1 ml aliquots of serum using a standard radioimmunoassay technique (Janssen Biotech). The detection limit of the assay was 1 ng/ml.[7]

Measurement of drug concentrations and calculation of pharmacokinetic data for the first phase were made by Janssen Pharmaceutical Ltd (AVP). Alfentanil plasma concentration-time data after the bolus dose were fitted to a one or two compartment model by using non-

Table 1 Pharmacokinetic data after single dose of alfentanil

	Median	*Range*
Dose given (mg/kg)	19·7	17·8–22·1
Half life (mins)		
α	7·6	2·0–38·1
β (elimination)	321	64·0–1251
Clearance (ml/kg/min)	0·87	0·4–9·62
Volume of distribution (ml/kg)	501	125–1039

linear least squares regression analysis and weighting by the inverse of the plasma concentration.[8]

PHASE II—INFUSION STUDY

The single dose pharmacokinetic data were used to calculate a suitable infusion regime. Because of the wide interpatient variability three schedules were evaluated with analysis of samples between each trial. Alfentanil was administered to a further 14 infants using the following regimes: a loading infusion of 15 µg/kg followed by 3 µg/kg/hour (five infants), a loading infusion of 20 µg/kg and 3 µg/kg/hour (five infants), and 20 µg/kg followed by 5 µg/kg/hour (four infants). The loading dose was given by continuous infusion over 30 minutes by a syringe driver (Model SP44 or IP5, Vickers Medical). Continuous monitoring of heart rate, blood pressure, and transcutaneous oxygen tension was performed as previously. Samples (0·4 ml) were taken before and at the completion of the loading infusion and twice daily for 48 hours or until muscle relaxants were discontinued when the alfentanil infusion was also stopped. The samples were handled as above. Serum alfentanil was again measured by radioimmunuoassay.

This study was approved by the district ethics committee.

Results

PHASE I—SINGLE DOSE STUDY

The median gestational age of the 22 infants was 30 weeks (range 25–36 weeks) and birth weight 1343 g (690–4084 g). Twelve infants were studied in the first 24 hours after birth, five at between 25 and 48 hours, and five between 49 and 96 hours. There were seven girls. At the time of sampling 16 infants had a serum creatinine concentration >100 µmol/l, three were jaundiced (bilirubin >150 µmol/l), and four had dopamine infusions running. One child was macrosomic after a pregnancy complicated by diabetes mellitus. Subsequently, four infants still required added oxygen at 28 days and four died. No deaths occurred during the period of data collection or were felt to be related to the administration of alfentanil.

The median dose of alfentanil given was 19·8 µg/kg (range: 17·8–22·1). The median alfentanil concentration five minutes after injection was 66 ng/ml (range: 20–606 ng/ml) (fig 1). Wide difference between patients were noted in terms of peak serum concentration and rate of decay, and in all related pharmacokinetic variables. Basic pharmacokinetic data are shown in table 1. No association between birth weight, gestation, age at testing, or gender and elimination half life or clearance could be demonstrated. Injection over two minutes was associated with a transient fall in blood pressure, heart rate, and arterial oxygen tension. These data have been reported previously.[9]

PHASE II—INFUSION STUDY

The results of the infusion studies are shown in table 2. Concentrations after loading with 15 µg/kg infusion at 3 µg/kg/hour were frequently unsatisfactory. After 20 µg/kg alfentanil given over 30 minutes followed by 5 µg/kg/hour satisfactory concentrations were obtained without significant change in cardiorespiratory variables. No significant accumulation of alfentanil was observed (fig 2).

Discussion

Before the administration of potent opioid

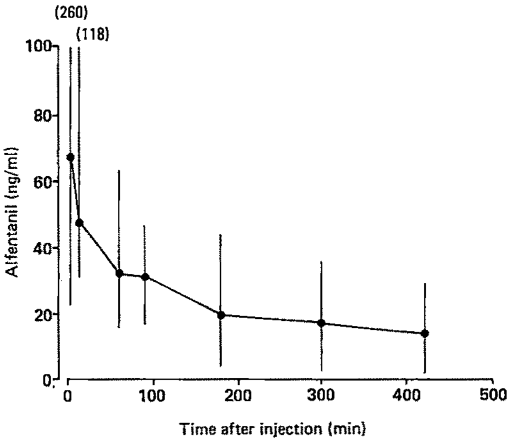

Figure 1 Alfentanil concentrations after single dose. Values shown are median, 10th and 90th percentiles.

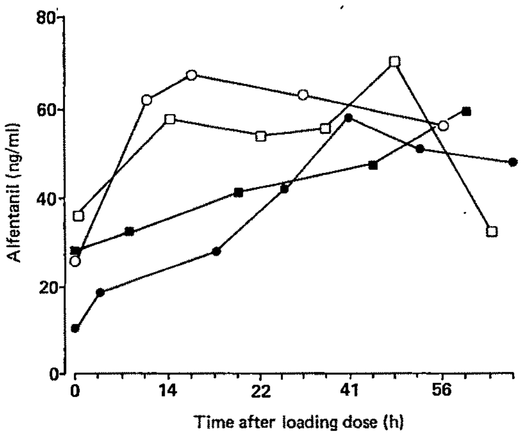

Figure 2 Alfentanil concentrations during infusion (four patients).

Table 2 Alfentanil concentrations during infusion

Loading dose (µg/kg)	*Infusion rate (µg/kg/hour)*	*No of subjects (No of samples)*	*Alfentanil concentration*	
			After loading dose: median (range)	*During infusion: median (range)*
15	3	5 (35)	18·5 (18–26)	17·8 (1–104)
20	3	5 (19)	20·0 (10–41)	29·0 (6–76)
20	5	4 (24)	27·0 (11–36)	54·5 (7–73)

drugs to sick preterm infants receiving mechanical ventilation becomes mandatory, it is necessary to collect background pharmacokinetic data and to demonstrate that such medication is both efficacious and safe. Clinical monitoring of frequency of administration of sedatives, such as chloral hydrate, may rapidly produce undesirably high levels (L Harris, presentation to Paediatric Research Society, October 1988), and there is no evidence that clinical monitoring of pethidine or morphine is any better. The routine adminstration of muscle relaxants, as on our unit, removes any possibility of clinical monitoring.

There was great variability in the handling of alfentanil in our study. Peak serum concentrations and clearance varied widely between subjects. This variability has been noted in pharmacokinetic studies of fentanyl[6 10] and morphine.[11] It is difficult to extrapolate from these studies, however, because all were performed in infants undergoing surgery, frequently for congenital heart disease, at varying postnatal ages. Compared with both older children and adults,[12 13] preterm infants have reduced clearance, prolonged elimination half life, and a greater volume of distribution of alfentanil.

The sick preterm newborn infant has multisystem illness with immature hepatic and renal function. Opiates are metabolised by the non-specific monoamine oxidases present in liver. Inactive metabolites are then excreted in the urine. Variation in hepatic and renal function may therefore exert a significant effect upon the metabolism of alfentanil. In this study no relation was observed between broad markers of hepatic and renal function (serum bilirubin and serum creatinine, respectively) and elimination half life or clearance. Bilirubin is only a crude indicator of liver function and clearly does not identify inadequate hepatic degradation of the drug.

In adults concentrations of alfentanil between 35 and 50 ng/ml are recommended for sedation during mechanical ventilation, although for analgesia during surgery concentrations of 200 ng/ml and higher are recommended. Although the regimen described in this paper achieved adequate sedative concentrations of alfentanil, it is important to remember that these levels are not analgesic for adults. The insertion of chest drains and other painful procedures must still be covered by adequate local and perhaps parenteral analgesia.

This study shows that it is possible to administer alfentanil without appreciable cardiovascular effects and with no accumulation over a 48 hour period. As both hepatic and renal function improve at the same time as the preterm child recovers from hyaline membrane disease one might expect improved opioid clearance and elimination. One study had observed shortening of elimination half life and increasing clearance of a similar drug, sufentanil, in infants returning for a second operation.[14] These improvements in drug handling are, however, not yet predictable and further studies are necessary to establish their time course. As yet, routine monitoring of serum concentrations for any of the commonly used analgesics is not practicable with current methodologies. The serum concentrations in this study are unlikely to lead to toxicity but low concentrations, and therefore inadequate treatment, are possible.

Although we have demonstrated that alfentanil infusions are possible in sick preterm infants, we have not established that adult equivalent sedative doses are effective in reducing the stress of mechanical ventilation, nor have we established that such a reduction of stress is beneficial. Further studies are in progress to establish these points.

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Calcium and phosphorus solubility in neonatal intravenous feeding solutions

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Abstract

The limited solubility of calcium and phosphorus in standard parenteral nutrition formulations has restricted the ability to provide sufficient minerals to preterm infants to prevent substrate deficient metabolic bone disease. We determined the solubility limits of calcium and phosphorus in a total of 160 formulations under carefully controlled conditions. By increasing the concentrations of dextrose, amino acids, and by using Addiphos instead of 8·7% dipotassium hydrogen phosphate as the phosphorus source, higher concentrations of both calcium and phosphorus were held in solution. This should permit the delivery of increased concentrations of these minerals at rates which approximate fetal accretion.

Metabolic bone disease is common in sick preterm infants.[1] The cause is multifactorial; inadequate calcium and phosphorus intake is a major factor.[2] The in utero accretion rate of calcium and phosphorus approximates 2·5–3·0 and 2·0–2·5 mmol/kg/day respectively in the third trimester.[3 4] During intravenous alimentation, the intake of these minerals is frequently restricted to about 1·0 mmol/kg/day[5] as it is not possible to deliver adequate amounts intravenously without exceeding their solubility limits.[6]

We wished to test whether it was possible to quantify the solubility limits of calcium and phosphorus in a range of neonatal intravenous feeding solutions with a view to maximising this solubility.

Methods

A total of 160 different amino acid/dextrose/mineral solutions were prepared and systematically tested for precipitation. The solutions contained all the constituents normally used in neonatal parenteral nutrition, with the exception of intravenous fat solution and fat soluble vitamins, as these usually come into contact with the single bag formulation immediately before infusion.

Each solution was individually formulated in the pharmacy department of Charing Cross Hospital under standard conditions; 200 ml of each was prepared. The concentrations of amino acid solution (Vamin 9 glucose, KabiVitrum), dextrose, calcium (10% calcium gluconate), and phosphorus (either as 8·7% dipotassium hydrogen phosphate or Addiphos, KabiVitrum) were varied to a protocol of increasing concentrations of calcium and phosphorus. The remaining constituents were added in concentrations as used clinically.

The solutions were prepared based on the assumption that a neonate on parenteral nutrition would receive in the order of 150 ml/kg/day (130 mg/kg/day test solution and 20 ml/kg/day of intravenous fat solution). The composition/kg/day of the test solutions was: sodium 3·9 mmol (3·0 mmol/100 ml), potassium 2·6 mmol (2·0 mmol/100 ml), trace elements as Ped-El (KabiVitrum) 4 ml (3·1 ml/100 ml), water soluble vitamins as Solivito N (KabiVitrum) 0·65 ml (0·5 ml/100 ml), magnesium in the amino acid and trace element preparations, 0·14–0·12 mmol (0·11–0·16 mmol/100 ml), and sodium heparin at 1 U/ml. Calcium and phosphorus were added in molar ratio of 1·2:1·0. The amount of these minerals and the amino acid and trace element solutions were included in all calculations. Additional phosphorus was supplied as either 8·7% dipotassium hydrogen phosphate or Addiphos, which is a proprietary preparation. This contains monobasic potassium phosphate 170·1 mg, sodium phosphate 133·5 mg, potassium hydroxide 14·0 mg, sorbitol 1·0 mg, and water for injection 1 ml. In total there is 15 mmol potassium, 15 mmol sodium, and 20 mmol phosphate in 10 ml.

During preparation of the solutions the environmental air was passed through a 0·3 micron filter. Volumes were measured to the nearest 0·01 ml and the solution was shaken and thoroughly mixed before the next constituent was added. The 200 ml minibags were placed in an incubator at 32°C and examined for the presence of precipitation by a single observer at 24 and 48 hours under polarised light. The upper limit of solubility was defined by the maximum concentration of calcium and phosphorus that could be added to any solution without precipitation occurring by 24 hours. The effect on the solubility limits of these solutions of omitting sodium heparin, of adding extra magnesium (equivalent to 0·4 mmol/kg/day or 0·3 mmol/100 ml), of omitting the trace elements, and alternating the calcium and phosphorus content with 10% calcium gluconate and either 8·7% dipotassium hydrogen phosphate or Addiphos was assessed. The pH and titratable acidity of 16 solutions, at the upper limit of calcium and phosphorus solubility, were determined assuming that 130 ml/kg/day of the solution was infused. The titratable acidity was measured by titrating a known volume of each solution to a pH of 7·4 using 0·025 M sodium hydroxide.

Results

Of 160 different solutions formulated, the effects of changing the amino acid or dextrose concentrations, and changing the phosphorus source from 8·7% dipotassium hydrogen phosphate to Addiphos of the 16 solutions which approximate to the limits of solubility are shown in figs 1 and 2.

For any given concentration of Vamin 9 glucose or dextrose, approximately twice as much calcium and phosphorus was held in solution if Addiphos was used instead of 8·7% dipotassium hydrogen phosphate, but at the expense of increasing titratable acidity. Completed formulations, at the upper limit of calcium and phosphorus solubility had a pH of between 5·73 and 5·88 when using Addiphos and 5·77 and 6·16 when using 8·7% dipotassium hydrogen phosphate. The pH of Addiphos is 7·02 and that of 8·7% dipotassium hydrogen phosphate 8·8. Assuming that the maximum amounts of calcium and phosphorus were delivered together with the amino acid solution at a rate of 40 ml/kg/day, the total potential acid load expressed as the titratable acidity in mmol/kg/day reached a maximum of 5·60 when using Addiphos and 3·85 when using 8·7% dipotassium hydrogen phosphate. The solubility limits were not significantly effected by omitting either the sodium heparin or the trace element preparation. Increasing the magnesium content to a maximum of 0·3 mmol/100 ml by the addition of 50% magnesium sulphate decreased the amount of calcium and phosphorus that could be held in solution by 9%.

Discussion

The solubility of calcium and phosphorus decreases when the pH rises,[7] the amino acid concentration falls,[6 7] there is a rise in the environmental temperature,[7] calcium is added before phosphorus,[8] or calcium chloride is used in place of calcium gluconate.[9] Previous attempts at documenting the solubility limits of calcium and phosphorus in neonatal parenteral solutions have been criticised[10] because of the failure to include magnesium, the restriction of environmental temperatures to 25°C, and the lack of definition in the accuracy and calibration of volume measurements.[9] We believe that this study overcomes these objections. Magnesium was included in all formulations over a range of concentrations; solutions were tested at 32°C after 24 hours, which is a higher temperature than quoted in most published series, and which exceeds the environmental temperature of the average neonatal unit. The use of relatively large volumes of the test solutions ensured that the volumes of some relatively concentrated constituents, such as Addiphos and 8·7% dipotassium hydrogen phosphate, could be measured with a high degree of accuracy. Calcium and phosphorus was added at a ratio of 1·2–1·0 as published evidence suggests that this is the optimal fetal accretion ratio.[3]

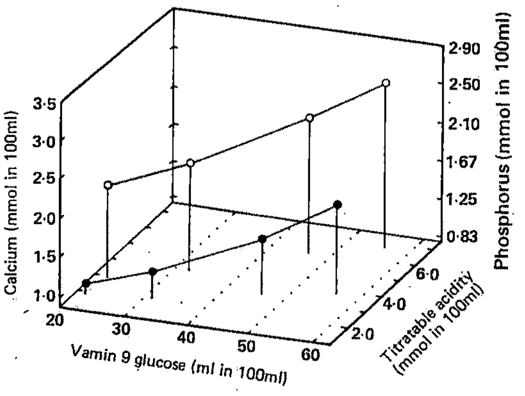

Figure 1 Changes in calcium and phosphorus content and titratable acidity of intravenous solution in 100 ml when varying the amino acid concentration in 10% dextrose. Dipotassium hydrogen phosphate (●) and Addiphos (○) as phosphorus source.

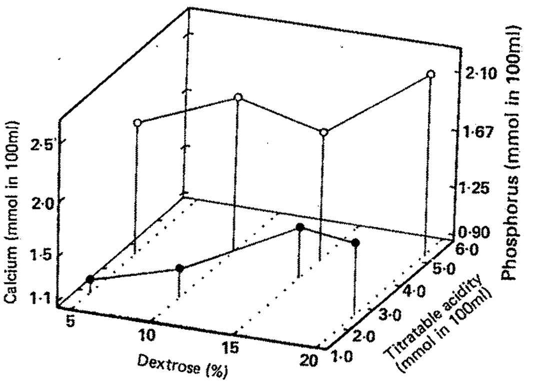

Figure 2 Changes in calcium and phosphorus content and titratable acidity in 100 ml of intravenous solution when varying the concentration of dextrose solutions in 30% Vamin 9 glucose. Dipotassium hydrogen phosphate (●) and Addiphos (○) as phosphorus source.

The upper limit of solubility of calcium phosphate is prone to a small random variation. This is probably due to the variation in the pH of some of the constituents, particularly dextrose which can vary from 3·5 to 5·5.[11] Our data would suggest that in clinical practice the maximum calcium and phosphorus concentration to be used should not exceed near 80% of the theoretical maximum for any given formulation because of the above variables. Figure 1 shows that by increasing the amino acid concentration, calcium and phosphorus solubility also increased but at the expense of an increased titratable acidity and therefore a potential hydrogen ion load.

The severity of metabolic bone disease in preterm infants fed intravenously is reduced if the calcium and phosphorus intake is increased.[12] Calcium and phosphorus content can be increased by using a slightly more acidic source of phosphorus during the formulation.

We thank Mr Nigel Edwards, principal pharmacist and his staff at Charing Cross Hospital for support.

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Acid-base state of the preterm infant and the formulation of intravenous feeding solutions

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Abstract

An acidic intravenous source of phosphorus (Addiphos) was compared with dipotassium hydrogen phosphate in 25 preterm infants to study acid-base state. Eight infants were given either Addiphos or dipotassium hydrogen phosphate alternately for 48 hour periods and similar amounts of calcium and phosphorus were delivered. There were no significant differences in calcium and phosphorus intake, calcium and phosphate plasma concentrations, or acid-base state between study periods on the two solutions. Seventeen infants were given the two solutions alternately for 72 hour periods; Addiphos was used to increase the amounts of calcium and phosphorus being delivered. Calcium and phosphorus intake was decreased on dipotassium hydrogen phosphate, but Addiphos significantly increased calcium and phosphorus intake and plasma calcium and phosphate concentrations. It also lowered the pH of the urine and raised the titratable acidity. Acid-base state, however, was not significantly different.

It is therefore possible to increase intake of calcium and phosphorus in preterm infants without causing a significant metabolic acidosis.

Metabolic bone disease is common in the sick preterm infant on intravenous alimentation.[1] An increased intravenous mineral intake decreases the incidence of this disorder.[2] In the third trimester the in utero accretion of calcium and phosphorus approximates 2·5–3·0 and 2·0–2·5 mmol/kg/day respectively.[3] Some authors have claimed that it is not possible to deliver comparable amounts of mineral in an intravenous solution at a physiological pH (7·4) because of dibasic calcium-phosphate precipitation.[4] Few parenteral solutions have a pH near 7·4; the pH of 5% dextrose, for example, varies between 3·5 and 5·5,[5] and intravenous feeding solutions themselves are invariably mildly acidic.[6]

The calcium and phosphorus content of an intravenous feeding solution can be increased by using a mildly acidic source of phosphorus during formulation in vitro, but at the expense of increasing the titratable acid load.[7] We wished to test the hypothesis that an increase in hydrogen ion load would enable calcium and phosphorus to be infused at in utero accretion levels, and yet not have any deleterious affect on the acid-base state of the preterm infant.

Patients and methods

Twenty five preterm infants who were admitted to a single neonatal intensive care unit and who required both ventilatory support and parenteral nutrition were studied. The prescription and formulation of parenteral nutritional solutions were assisted by modification of a pre-existing computer protocol.[8] Calcium was supplied as 10% calcium gluconate in the trace element preparation (Ped-El, KabiVitrum) and in the amino acid solution (Vamin 9 glucose, KabiVitrum). Phosphorus was supplied in the trace element preparation (Ped-El) and as either 8·7% dipotassium hydrogen phosphate (pH 8·8), or as Addiphos (KabiVitrum, pH 7·02).

The infants were randomly allocated to receive initially either 8·7% dipotassium hydrogen phosphate or Addiphos at 8 days of life. This was then alternated every 48 hours (part I) or 72 hours (part II). The interval during which the phosphorus source was kept constant was termed a 'study period'. At the end of each study period the overall acid-base state of the infant was assessed by calculating the mean blood pH, carbon dioxide pressure, standard bicarbonate, and base excess measurements during the last 24 hour period. The urinary pH and titratable acidity/mmol creatinine were determined in a random urine sample at the end of each study period.

Plasma calcium phosphate concentrations were assayed every 48 hours using approved methods on a Parallel Analyser (American Monitor Corporation). Blood gas estimations were assayed at least every four hours while the infant received assisted ventilation, using an IL-1302 blood gas analyser (Instrumentation Laboratories). The pH of each urine specimen voided was measured to one decimal place using pH indicator strips (British Drug Houses). The urine titratable acidity was determined by titrating a known volume of urine to pH of 7·4 using 0·025M sodium hydroxide.

PART I

This part of the study was designed to compare the affect of using either 8·7% dipotassium hydrogen phosphate or Addiphos on the acid-base state while delivering similar amounts of calcium and phosphorus. The eight infants studied had a median birth weight of 1080 g (range 870–1640 g) and a median gestational age of 29·0 weeks (range 27·0–29·5). There were 30, 48 hour study periods using 8·7% dipotassium hydrogen phosphate (group 1) and 29 using

Table 1 Part I: comparison of intravenous intake, plasma values, and acid-base state in the 48 hour study periods in which the infants received either 8·7% dipotassium hydrogen phosphate or Addiphos to deliver equal amounts of calcium and phosphorus. Results are mean (SD)

	Group 1 (n=30)	*Group 2 (n=29)*	*p Values*
Phosphorus source	Dipotassium hydrogen phosphate	Addiphos	
Intake:			
Calcium (mmol/kg/day)	1·27 (0·35)	1·28 (0·30)	NS
Phosphorus (mmol/kg/day)	1·22 (0·31)	1·26 (0·27)	NS
Dextrose (MJ/kg/day)	0·21 (0·03)	0·21 (0·04)	NS
Vamin 9 glucose (ml/kg/day)	44·30 (7·04)	46·90 (7·01)	NS
Lipid (g/kg/day)	1·99 (1·01)	2·12 (1·20)	NS
Plasma:			
Calcium (mmol/l)	2·35 (0·18)	2·40 (0·15)	NS
Phosphate (mmol/l)	1·54 (0·40)	1·48 (0·13)	NS
Blood:			
pH	7·32 (0·04)	7·33 (0·05)	NS
Carbon dioxide pressure (kPa)	6·07 (0·80)	6·00 (0·80)	NS
Standard bicarbonate (mmol/l)	22·60 (1·08)	22·60 (1·07)	NS
Base excess (mmol/l)	−2·70 (4·06)	−2·50 (4·04)	NS
Urine:			
pH	5·25 (0·39)	5·20 (0·81)	NS
Titratable acidity (mmol/mmol creatinine)	6·12 (2·55)	5·54 (2·02)	NS

Table 2 Part II: comparison of intravenous intake, plasma values, and acid-base state in the 72 hour study periods in which the infants received either 8·7% dipotassium hydrogen phosphate or Addiphos to deliver significantly increased amounts of calcium and phosphorus. Results are mean (SD)

	Group 3 (n=35)	*Group 4 (n=32)*	*p Values*
Phosphorus source	Dipotassium hydrogen phosphate	Addiphos	
Utake:			
Calcium (mmol/kg/day)	0·67 (0·25)	1·63 (0·48)	<0·001
Phosphorus (mmol/kg/day)	0·60 (0·13)	1·38 (0·40)	<0·001
Dextrose (MJ/kg/day)	0·23 (0·07)	0·22 (0·07)	NS
Vamin 9 glucose (ml/kg/day)	35·00 (7·03)	37·00 (5·90)	NS
Lipid (g/kg/day)	2·20 (1·38)	2·24 (1·23)	NS
Plasma:			
Calcium (mmol/l)	2·33 (0·20)	2·47 (0·23)	<0·05
Phosphate (mmol/l)	1·30 (0·33)	1·60 (0·32)	<0·005
Blood:			
pH	7·32 (0·05)	7·30 (0·05)	NS
Carbon dioxide pressure (kPa)	6·27 (0·87)	6·13 (0·76)	NS
Standard bicarbonate (mmol/l)	23·29 (2·3)	22·07 (1·80)	<0·05
Base excess (mmol/l)	−1·50 (4·55)	−2·15 (6·45)	NS
Urine:			
pH	5·56 (0·71)	5·22 (0·47)	<0·02
Titratable acidity (mmol/mmol creatinine)	5·49 (1·89)	8·06 (2·25)	<0·001

Addiphos (group 2). Amino acid solution was infused to a maxium rate of 50 ml/kg/day.

PART II

This part was designed to test the affect on the acid-base state using Addiphos in order to deliver increased calcium and phosphorus. Seventeen infants were studied with a median birth weight of 1120 g (range 750–1500 g) and a median gestational age of 28·5 weeks (range 24·0–30·0). The amino acid infusion rate was reduced to a maximum of 40 ml/kg/day because of concern about hyperphenylalaninaemia, which had been reported coincidentally during the conduct of this study. This change altered the solubility limits of calcium and phosphorus thereby limiting the amount of calcium and phosphorus that could be delivered if 8·7% dipotassium hydrogen phosphate was used.[6] There were 35, 72 hour study periods using 8·7% dipotassium hydrogen phosphate (group 3) and 32 using Addiphos (group 4).

The last 24 hours of each study period in both parts were analysed and each period was treated as an independent event. The results were expressed as mean (SD). Tests of significance were performed using the Student's *t* test.

The study was approved by the hospital ethics committee.

Results

In part I, there was no significant difference between groups 1 and 2 for overall calcium and phosphorus infusion, plasma calcium and phosphate concentrations, or the indices of acid-base state in either blood or urine (table 1).

In part II, the calcium and phosphorus intake was decreased in group 3 when 8·7% dipotassium hydrogen phosphate was used as the source of phosphorus. When using Addiphos, however, the amounts of calcium and phosphorus infused were significantly increased as were both the plasma calcium and phosphate concentrations (table 2). There was no significant difference in blood acid-base state between groups 3 and 4, with the exception of a small difference in the calculated standard bicarbonate, which remained within the reference range of 21–30 mmol/l. During the study period in which Addiphos was used, the pH of the urine was significantly lower ($p<0·02$) and titratable acidity higher ($p<0·001$) than when 8·7% dipotassium hydrogen phosphate was used.

Discussion

We are not aware of a comparable study in which the ability of a preterm infant to tolerate an increased hydrogen ion infusion load was tested. Although metabolic acidosis is a recognised complication of parenteral nutrition, the incidence has been reduced by using crystalline amino acid preparations when compared with the earlier protein hydrolysate solutions.[9]

An increased hydrogen ion load is buffered in blood and excreted in urine. Urinary pH reflects the acidity, but not the buffering capacity of the urine. Urinary titratable acidity is a more appropriate assessment of the total hydrogen ion excretion. Measuring the pH from a random urine sample introduces further inaccuracies. Obtaining accurate 24 hour urine collections repeatedly can be difficult, particularly in infant girls. We felt it appropriate to measure urinary titratable acidity in relation to creatinine excretion, in order to obtain comparative results.

There was no change in acid-base state in the infants given Addiphos rather than 8·7% dipotassium hydrogen phosphate despite the increased hydrogen ion infusion load. The amount of calcium and phosphorus that could be delivered is increased if the rate of amino acid infusion is increased as the extra hydrogen ion load alters their solubility. High infusion rates of amino acids may, however, be associated with hyperphenylalaninaemia.[10] The amount of calcium and phosphorus that could be infused in part II using 8·7% dipotassium hydrogen phosphate as the phosphorus source was less compared with Addiphos, which is the more acidic agent. The increased hydrogen ion load associated with the use of Addiphos did not produce a metabolic acidosis, and the extra load was successfully excreted in the urine. Whether or not the more prolonged use of relatively acidic, high calcium, high phosphorus parenteral nutrition protocol could be associated with nephrocalcinosis will have to be borne in mind in future studies.

These studies demonstrate that it is possible to increase significantly the intravenous intake of calcium and phosphorus in the preterm infant by using an acidic source of phosphorus without causing a significant metabolic acidosis.

We thank the nursing staff of the neonatal intensive care unit at the West London Hospital for their help.

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Renal solute load in preterm infants

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Abstract

We performed metabolic balance studies in 77 preterm infants fed on human milk or adapted formulas, to determine the renal solute load and compared it with the potential renal solute load estimated by the composition of the diet or the urine according to Ziegler's, Bergman's, and Shaw's calculations. The renal solute load found in preterm infants was lower than that observed in young full term infants. Although all calculations predicted the renal solute load well, the equation proposed by Ziegler and Fomon from dietary protein and electrolyte load appears to be the simplest to calculate renal solute load accurately in preterm infants.

Renal solute load consists of the metabolic end products, especially nitrogenous compounds and electrolytes, that must be excreted by the kidneys. These solutes arise from endogenous sources and from the diet.

Prediction of renal solute load is important because renal function is not fully developed in the neonatal period, particularly in preterm infants. Indeed because of their limited renal concentrating ability, immature infants can develop negative water balance when fed on formulas with a high renal solute load, or if they are given a low water intake, or develop increased extrarenal losses of water.

Ziegler and Fomon derived a simple method to predict renal solute load from dietary protein and electrolyte intake in growing full term infants of 4000 to 7000 g fed cows' milk.[1] Because some of the assumptions in their calculations are not appropriate for growing infants with immature renal function Bergman, Ziegler, and Fomon published another method for predicting the renal solute load in growing preterm infants.[2]

Shaw *et al* have described the estimation of renal solute load in growing preterm infants from urinary excretion of urea and electrolytes, assuming a retention of nitrogen, sodium, and potassium based on rates of intrauterine accretion.[3]

The aim of this study was to compare the potential renal solute load (calculated from the equations proposed by these authors) and the measured renal solute load in preterm infants fed on human milk or different formulas during metabolic balances.

Patients and methods

Metabolic balance studies were carried out in 77 male low birthweight infants of appropriate weight for gestational age; 16 were fed on human milk and 61 on adapted formulas. There were no significant differences between the two groups in mean (SD) birth weight (1788 (199) compared with 1786 (204) g), gestational age (33 (2) compared with 33 (2) weeks), age, and weight at beginning of the study (21 (7) compared with 20 (7) days) and (2056 (230) compared with 2025 (277) g).

None of the infants had any congenital abnormality or medical problems at the time of the studies.

Informed consent was obtained from the parents.

NUTRITIONAL BALANCE

The infants were fed by nasogastric tube or by bottle when possible. The approximate volume of human milk or formula needed for the entire study was pooled, carefully mixed, and an aliquot was taken for chemical analysis.

The nutritional balance consisted of precise measurements of intake and output over a three day period, during which the infant lay on a metabolic bed placed within an incubator as previously described.[4] The intake of feed was measured volumetrically. Stool and urine were collected separately.

During each three day period urine was collected into a bottle containing 2 ml 10% thymol solution in isopropanol and kept cold in ice water. At the end of each 24 hours, urine collection was stored at −20°C. After thawing analyses were performed on the three day pooled urine collection.

ANALYSIS

From aliquots of prepared milk and urine, nitrogen was determined by the standard micro-Kjeldhal method, sodium and potassium were measured by a flame photometer, and chloride by mercuric titration.[5]

Energy content of human milk was estimated at 0·28 MJ/100 ml while that of infant formulas was as stated by the manufacturers and the range varied between 0·26 and 0·33 MJ/100 ml.

Urine osmolality was determined by the Fiske apparatus, creatinine was determined using a modification of Jaffe's method, ammonia by the Berthelot reaction, and uric acid, calcium, and phosphorus by methods used for their measurement in milk.[5]

CALCULATION OF POTENTIAL RENAL SOLUTE LOAD

The true renal solute load was calculated from

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urinary volume and urinary osmolality. Potential renal solute load, calculated as proposed by Ziegler and Fomon, was defined as the sum of nitrogen, sodium, potassium, and chloride in the intake. Each gram of dietary protein was considered to yield 4 mmol of renal solute load.

According to the equation proposed by Bergman *et al*, the potential renal solute load was also evaluated from the sum of dietary nitrogen, sodium, potassium, chloride, and phosphate. These workers assumed that all dietary protein is metabolised and all the metabolic products are presented to the kidney for excretion, so each gram of dietary protein was considered to yield 5·7 mmol of renal solute load.

Because the growing infant incorporates into new tissue a portion of those substances present in the diet (protein, potassium, phosphate, etc), which in the non-growing subject would be presented to the kidney for excretion, an 'osmolar equivalent of weight gain' was removed from the calculated potential renal solute load. A theoretical estimate of the size of this sparing effect, based on the body composition of the newborn infant, suggests an osmolar equivalent of weight gain of 0·9 mmol/g weight gain.[2]

Finally, the potential renal solute load was evaluated using Shaw's calculations, from the equation: total urine solute=(urine urea/mmol/day)×2(urinary Na+urinary K/mmol/day).[3] Urine urea and urine sodium (Na) and potassium (K) were calculated by subtracting from the intake a quantity assumed to be retained in growing preterm infants: 320 mg nitrogen/kg/day, 1 mmol/kg/day of sodium, and 0·7 mmol/kg/day of potassium.[3]

All values are expressed per kg body weight/day.

STATISTICAL ANALYSIS

The significance of the difference between the arithmetic means was evaluated using Student's *t* test. The association between potential renal solute load and measured renal solute load was analysed using the method of least squares and linear regression analysis.

Results

The table reports the results of metabolic balance studies in the two groups expressed as mean (SD)/kg body weight/day. Volume and energy intake were similar in the two groups.

Nitrogen intake in the formula fed group was significantly higher than in the group fed human milk because of the higher protein content of formula. The infants fed on human milk received an average of 424 mg nitrogen/kg/day corresponding to a net protein intake of 2·7 g/kg/day. Infants fed on formula received an average of 548 mg nitrogen/kg/day corresponding to a net protein intake of 3·4 g/kg.

Phosphorus intake was higher in formula fed infants than in the group fed on human milk. Urinary volume was similar in the two groups and represented about three quarters of the volume intake. Nitrogen excretion was significantly higher in formula fed infants and averaged 21% of the nitrogen intake in the group fed on human milk and 38% in the formula fed group. Distribution of total urinary nitrogen was 72% in urea, 10% in uric acid, 9% in ammonia, 5% in creatinine, and 2% undetermined in the group fed human milk and 76%, 10%, 9%, 4%, and 1% respectively in the formula fed group.

Results of metabolic balance studies in the two groups. Values are expressed as mean (SD)/kg body weight/day unless otherwise stated

	Type of milk	
	Human (n=16)	*Infant formula (n=61)*
	Intake	
Volume (ml)	194 (7)	193 (8)
Energy (MJ)	0·54 (0·42)	0·51 (0·71)
Nitrogen (mmol)	30·3 (2·3)	39·1 (8·4)
Sodium (mmol)	1·3 (0·1)	1·8 (0·6)
Potassium (mmol)	2·6 (0·2)	3·2 (0·4)
Chloride (mmol)	2·6 (0·2)	2·3 (0·6)
Phosphorus (mmol)	0·94 (0·06)	2·29 (0·29)
	Output	
Urine volume (ml)	141 (14)	140 (16)
Osmolality (mmol/kg)	102 (19)	143 (40)
Renal solute load (mmol)	14·4 (3·1)	19·7 (4·3)
Nitrogen (mmol)	6·4 (1·4)	14·9 (6·8)
Urea (mmol)	2·28 (0·76)	5·56 (2·97)
Creatinine (mmol)	0·11 (0·01)	0·12 (0·02)
Uric acid (mmol)	0·16 (0·02)	0·37 (0·03)
Sodium (mmol)	0·7 (0·6)	1·1 (0·7)
Potassium (mmol)	1·3 (0·3)	1·5 (0·6)
Chloride (mmol)	2·1 (0·7)	1·6 (0·7)
Calcium (mmol)	0·5 (0·2)	0·2 (0·1)
Phosphorus (mmol)	0·03 (0·03)	1·29 (0·52)
Ammonia (mmol)	0·6 (0·2)	1·3 (0·4)
Renal solute load/0·42 MJ	11·2 (2·4)	16·2 (3·9)
Weight gain (g)	15 (2)	15 (3)

As shown in fig 1, the contribution of urea to the renal solute load increased with increasing renal solute load.

Combined urinary excretions of sodium, chloride, and potassium accounted for approximately 60% of dietary intake in both groups (on average 63% in the group fed human milk and 57% in the formula fed group). The remainder of the renal solute load was almost constant.

There was a highly significant association (r=0·74; p<0·001) between potential renal solute load estimated by Ziegler and Fomon and

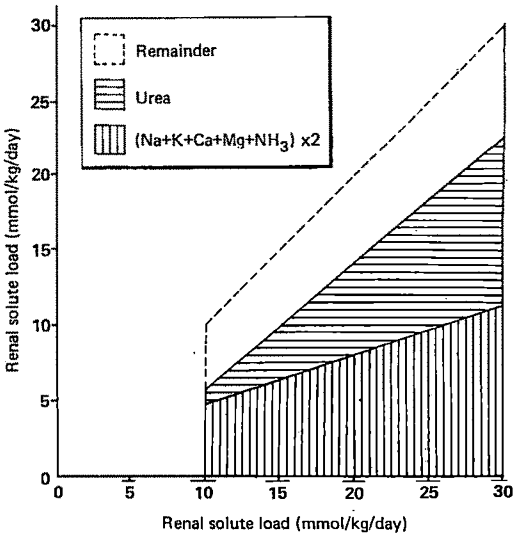

Figure 1 Composition of renal solute load. Na, sodium; K, potassium; Ca, calcium; Mg, magnesium; and NH_3, ammonia.

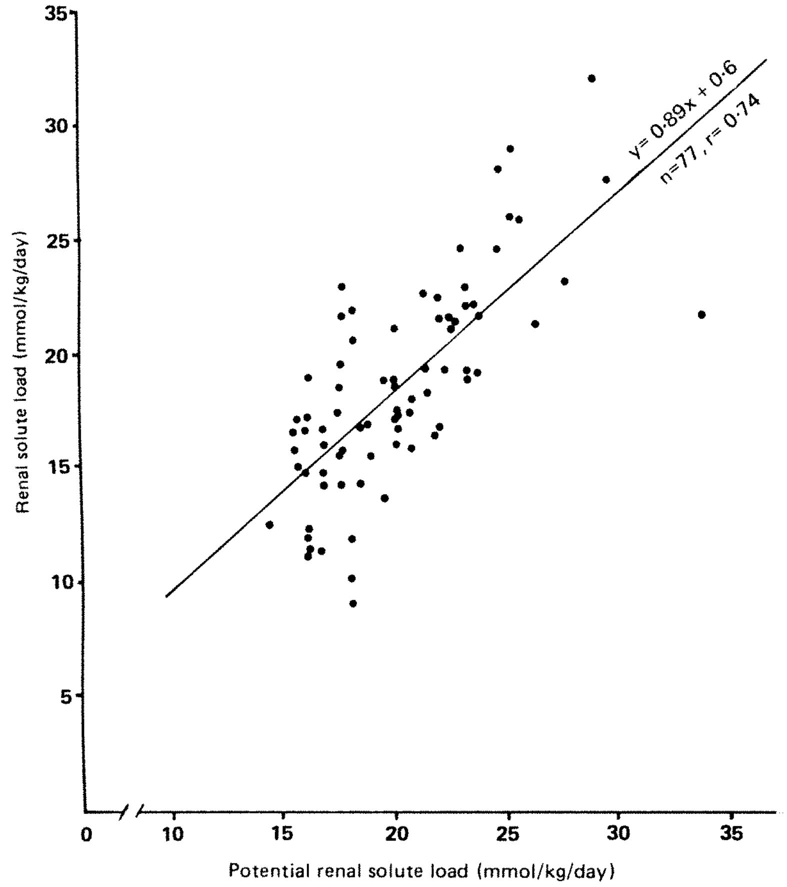

Figure 2 Relationship between the potential renal solute load estimated by Ziegler and Fomon[1] and true renal solute load found.

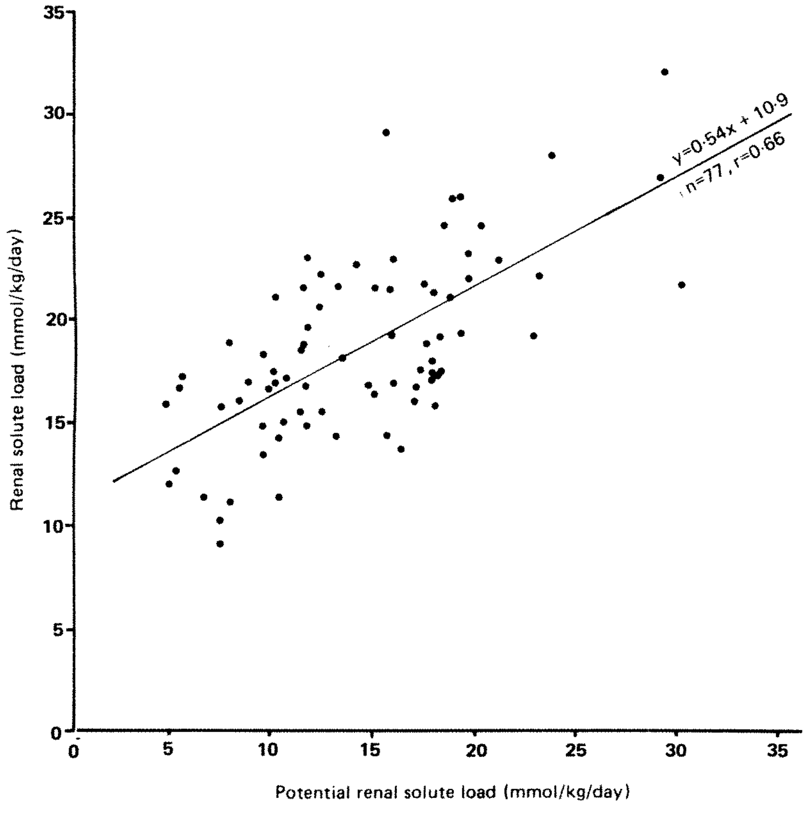

Figure 3 Relationship between the potential renal solute load estimated by Bergman et al[2] and true renal solute load found.

the measured renal solute load (renal solute load=0·89 potential renal solute load+0·6) (fig 2). There was also a significant relationship between the measured renal solute load and the potential renal solute load as estimated according to Bergman *et al* (r=0·66; p<0·001) (renal solute load=0·54 potential renal solute load+10·9) (fig 3); and as estimated according to Shaw (r=0·74; p<0·001) (renal solute load =0·6 potential renal solute load+10·5).

Discussion

Solute that must be excreted in the urine is referred to as the renal solute load and its main contributors are nitrogenous substances and electrolytes. The measured renal solute load in our infants ranged from 9 to 18·9 mmol/kg body weight/day in the group fed human milk and from 11·3 to 32·3 in the formula fed group. The renal solute load expressed/0·42 MJ energy intake was on average 11·2 and 16·2 mmol/kg body weight/day in both groups. These results appear lower than those observed in young infants.[6] This can be explained by the higher growth rate of preterm infants associated with a raised nitrogen and mineral retention rate. Mean urinary osmolar concentration was on average about 102 mmol/l (range 69–129) in the group fed human milk and 143 mmol/l (range 92–212) in the formula fed group. An estimate of renal solute load should be simple if it is to be useful in paediatric practice. The simplest calculation to predict renal solute load in preterm infants is that of Ziegler and Fomon because it is based on dietary intake of nitrogen and three major minerals: sodium, potassium, and chloride.

It is quite clear that the equation of Ziegler and Fomon (fig 2) not only produces results with a good correlation coefficient, but a slope that is fairly close to the line of equality. Thus across the range tested the equation is equally effective at predicting the true solute load. By contrast the data produced by using Shaw's equation (fig 4) give a similar correlation coefficient but a slope that is very different from zero and a fairly large constant value. This means that the formula is very inaccurate when used at the lower end of the scale: for example when the equation predicts a value of about 7 mmol/kg/day, the true value is likely to be about 14. The same criticism applies to Bergman's equation (fig 3) in which the slope is even shallower and the constant value slightly higher.

Bergman's calculation, which also considers the phosphorus intake, and the weight gain, and assumes that all dietary protein is excreted as urea, gave a less good estimation of the true renal solute load.

The ranges of urinary osmolality in our study appear particularly low. This could be due to high urinary volume and to low extrarenal water losses. Assuming that 10 ml/kg/day of water are retained for a normal growth of 15 g/kg/day by a preterm infant,[7 8] we estimated the extrarenal water lost at 43 ml/kg/day in both groups studied. This is represented in part by water lost in faeces, which is almost constant (5–10 ml/kg/day) and by insensible water losses that

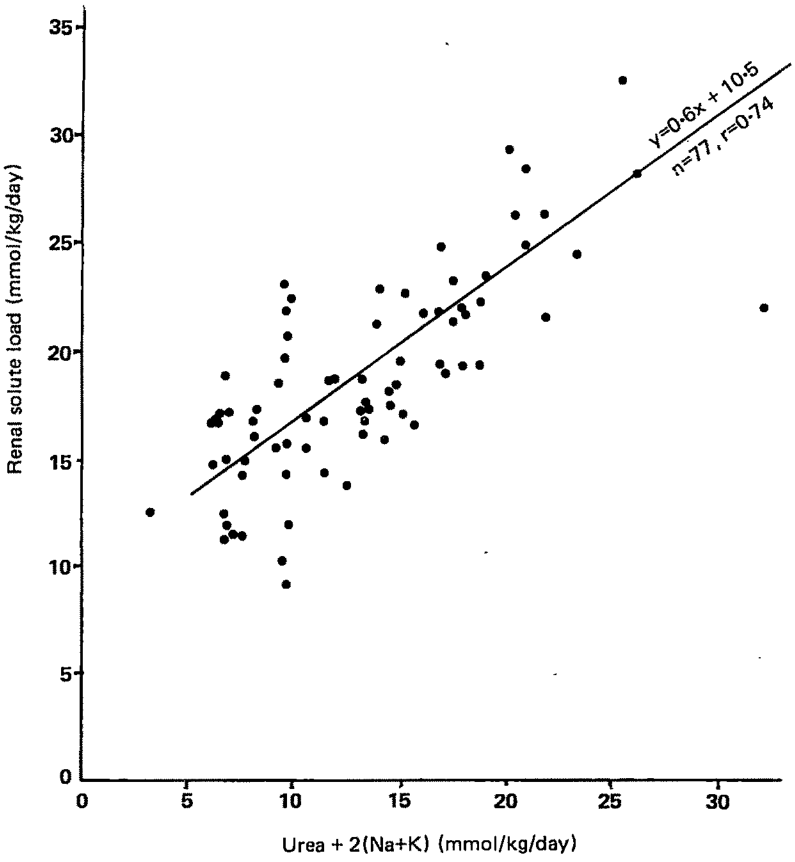

Figure 4 Relationship between the potential renal solute load estimated by Shaw et al[3] *and true renal solute load found.*

are variable and increase with decreasing body weight and gestational age.

The main conclusion of this study is that in healthy preterm infants fed on human milk and adapted formulas there is a relatively low renal solute load in comparison with older infants. This will not stress the urinary concentration mechanism if the extrarenal water losses are low and the urinary volume high.

The calculation proposed by Ziegler and Fomon to predict renal solute load in term infants appears to be a simple and a precise method for estimating renal solute load in healthy preterm infants.

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Ultrasound measurement of renal size in newborn infants

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Abstract

Renal measurements were made by ultrasonography in 560 healthy newborn infants. Three dependent variables (kidney length, kidney depth, and kidney area) were measured, together with three independent variables (birth weight, head circumference, and gestational age).

The SD of the measurement inconsistency was 0·13 cm for length, 0·078 cm for depth, and 0·32 cm^2 for area; the percentages of total variance caused by measurement inconsistency were 7·9, 9·5, and 7·5, respectively. There was a significant difference between right and left kidneys in length and depth for each independent variable. The left kidney increased more in length and less in depth than the right so that the areas remained similar as the scale of the variable increased. Kidney depth and area in boys were significantly larger than in girls, both dimensions increasing at equal rates.

Centile charts for each dependent variable by each independent variable were constructed by a non-parametric method.

To discover whether early postnatal ultrasound scanning of the urinary tract might show any evidence of maldevelopment or malfunction—in particular, ureteric reflux—a survey was undertaken of newborn infants delivered at the Princess Mary Maternity Hospital, Newcastle upon Tyne, between 1 January and 31 December 1986. As many of the babies as possible had renal ultrasound scans by a senior radiographer (EWH) between 48 and 72 hours after delivery. Permission for the examination was always sought from the mother, who was given an explanation—both verbally and in writing—of the purpose of the study, the technique to be used, and the steps that would be taken if anything abnormal was found. Approval was obtained from the Newcastle Health Authority ethics committee.

During the project we took the opportunity to measure as many kidneys as possible to try and compile a series of values that could be used in assessing normal (and ultimately, by comparison, abnormal) renal sizes in newborn infants. In this paper we describe the results of the measurements.

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Patients and methods

Of a total of 2764 live births during 1986, 1100 mothers (39·8%) were asked for permission to examine their babies. Twenty three refused, and in a further 15 the examination could not be conducted for technical reasons. A total of 1062 babies were examined. Because of a malfunction in the standoff accessory during the survey, renal measurements were obtained in only 565.

SCANNING TECHNIQUE

Babies were usually examined within one hour of a feed so that they would be soothed and relaxed; in many instances they were actually asleep. Apart from the sedative effect, the recent feed ensured a high urine output.

With the baby supine the bladder was scanned to assess its volume. The baby was then rotated into the prone position and after it had settled the kidneys were examined from the posterior aspect. This orientation was used uniformly for both kidneys in all babies even though it was occasionally necessary to make a baby more comfortable by placing it in an oblique or lateral position. The right kidney was usually examined first.

A Hewlett Packard 77020A sector scanner with 5 MHz short and medium focus transducers and a 3·5 cm standoff accessory was used.

The maximal length (superior to inferior perimeter), depth (superficial to deep perimeter), and area (best fit to circumferential perimeter in longitudinal section) of each kidney were measured twice using two separate frozen images. We also recorded the sex, head circumference, birth weight, and gestational age of the babies and whether they were singletons or multiple births.

Results

Five babies were excluded from the analysis (two had solitary kidneys, one had a horseshoe kidney, and in two only one kidney was measurable) leaving 560 babies for analysis.

MEASUREMENT ERROR

In a study of this kind two sources of error will be encountered: natural variation and measurement inconsistency. Because each kidney was measured twice in each dimension it was possible to estimate each source of variation separately. The natural variation is known as the 'between subject' variance and the measurement inconsistency as the 'within subject' variance. As there was little difference between the sexes their results were combined, as were the results for left and right kidneys. Table 1 shows the SD of the measurement inconsistency

Table 1 Renal size in newborn infants: measurement error and components of variance (total, right and left)

Dimension	*Between subject*	*Within subject*	*SD of measurement inconsistency*	*Percentage total variance because of measurement inconsistency*
Length (cm^2)	0·194	0·0168	0·13 cm	7·9
Depth (cm^2)	0·0580	0·00607	0·078 cm	9·5
Area (cm^4)	1·248	0·101	0·32 cm^2	7·5

and the percentage it contributes to the total variance of the measurements. Considering the difficulties of obtaining organ measurements by ultrasound scanning, particularly in small babies, accuracy was acceptably high.

GENERAL ANALYSIS

Three independent variables were used (birth weight, gestational age, and head circumference) to correlate with the three dependent variables (kidney length, kidney depth, and kidney area). A kidney measurement refers to the mean of the two replicates.

Table 2 shows the sex distribution and the means (SD) of the independent variables, and table 3 the means (SD) of the dependent variables.

Kidney size

Table 3 shows that the left kidneys were significantly longer and thinner than the right. Although the differences for length and depth are highly significant, the confidence intervals show that the size of these differences is small (about 1 mm).

Association between the means of left and right measurements (mean measurements) and the differences in measurements between sides (left–right) (difference measurements) and sex, birth weight, head circumference, and gestational age

After preliminary analyses, gestational age was abandoned as an independent variable because: the association with kidney size was similar to those of birth weight and head circumference, but generally weaker; it was an extremely skew and coarsely grouped variable so that associations were difficult to detect; and the measurement was likely to be inaccurate, thereby confusing the analysis.

Table 2 Renal size in 560 newborn infants: analysis of independent variables

Sex:	
Male	305
Female	255
Median (range) gestational age (weeks)	40 (33–42)
Mean (SD) birth weight (g)	3291 (517)
Mean (SD) head circumference (cm)*	34·8 (1·4)

*Data not available for 12 infants.

Mean measurements—measurements of depth and area were positively associated with birth weight and head circumference (p <0·001 in all cases). Sizes increased at a similar rate in both sexes though male kidneys were slightly larger than female; the difference was stronger with area (birth weight: p=0·001, head circumference: p=0·001) than with depth (birth weight: p=0·04, head circumference: p=0·06). Although significant, the size of the difference between boys and girls was small, roughly 0·6 mm.

Boys differed significantly from girls in the rate at which mean kidney length increased by both birth weight (p=0·03) and head circumference (p=0·01), boys being generally larger and increasing faster.

Difference measurements (always left – right)—There was no association between length difference measurements and birth weight, head circumference, or sex. For area and depth there was a marginally significant pattern with a tendency for the difference to decrease with increasing birth weight and head circumference in boys, and to increase in girls, though this did not apply to the association between depth and head circumference.

The significance of this finding is unclear. As with the mean measurements, however, the magnitude of the effects is too small to influence the broader assessment of kidney size in newborn infants.

Centile charts

In newborn infants attention is more likely to be drawn to a kidney that is suspiciously large than to one that is suspiciously small. Conditions such as cystic disease, duplex malformation, hydronephrosis, or neoplasm, are more common in the newborn than hypoplasia or dysplasia. In the construction of the centile charts, therefore, the maximum measurement of the right or left kidney was used. Separate charts for boys and girls were not constructed because this would have roughly halved the number of plots/chart and confused the calculation. Furthermore, the mean difference in measurement between the sexes was small, about the same as the SD of the measurement inconsistencies.

A recently introduced non-parametric method was used.[1] All the variables were adequately modelled by linear centiles; the intercepts were quadratic and the slopes were linear in the standard normal variate. The models were checked by comparing centile charts for the derived SD scores with those expected from

Table 3 Renal size in 560 newborn infants: analysis of dependent variables

Dimension	*Right: mean (SD)*	*Left: mean (SD)*	*95% Confidence interval of difference between left and right*	*p Value*
Length (cm)	4·21 (0·45)	4·32 (0·46)	0·08 to 0·14	<0·001
Depth (cm)	2·23 (0·27)	2·14 (0·23)	−0·10 to −0·07	<0·001
Area (cm^2)	6·48 (1·19)	6·44 (1·11)	−0·11 to 0·03	0·29

standard normal variables (MJR Healy, personal communication). The charts refer to the maximum of the means of two replicate measurements made on each kidney.

Centile charts showing 3, 10, 25, 50, 75, 90, and 97 centiles were constructed for kidney length and depth by birth weight and head circumference (figs 1–4). Charts for kidney area were also constructed but are not shown.

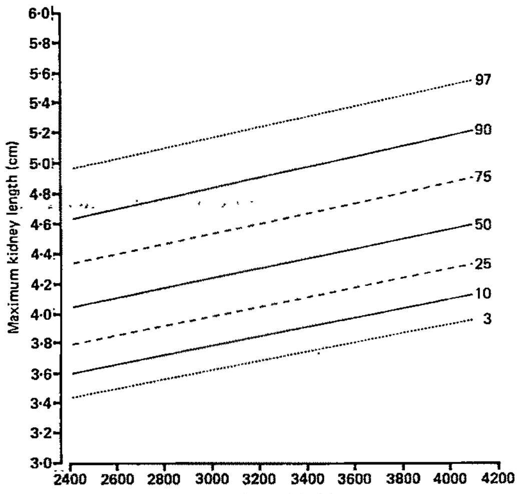

Figure 1 Centile chart: maximum kidney length by birth weight.

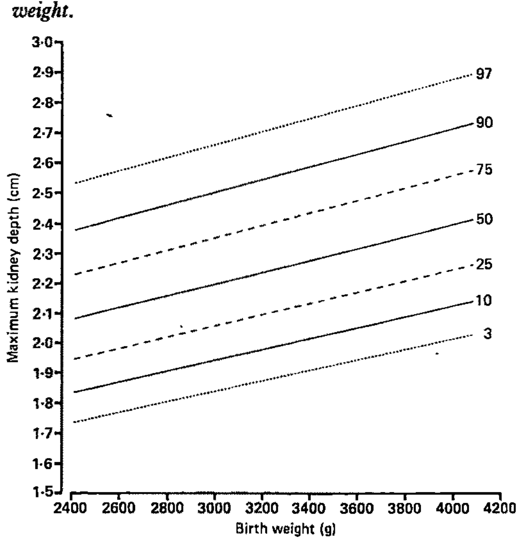

Figure 2 Centile chart: maximum kidney depth by birth weight.

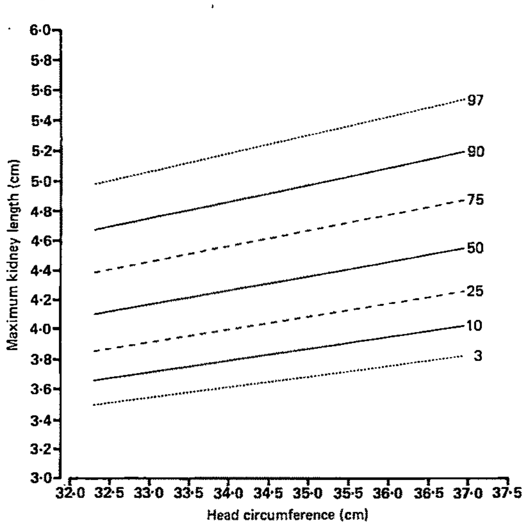

Figure 3 Centile chart: maximum kidney length by head circumference.

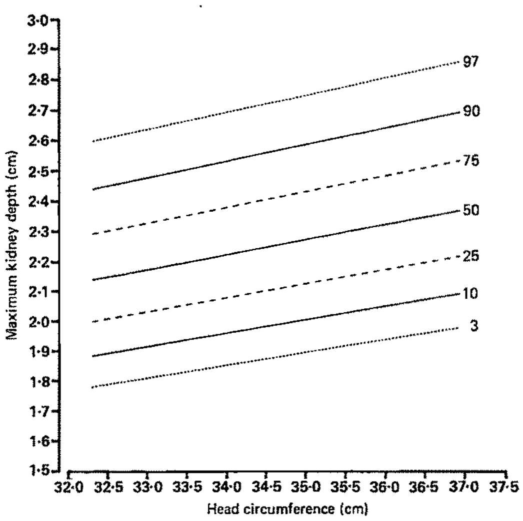

Figure 4 Centile chart: maximum kidney depth by head circumference.

Discussion

In a study of 100 unselected preterm and term infants renal length was measured by ultrasound scanning, compared with postmenstrual age, and a growth chart plotted.[2] In 20 of these infants a ratio between kidney length and crown-heel measurement was calculated and kidney size remained in proportion to the length of the infant even though the infant was either small or large for gestational age. In study of 36 newborn infants, renal volume and length were measured in order to obtain a mean figure.[3] There was no correlation with body weight, height, or surface area, but differences in length between right and left kidneys (similar to the current series) were noted, although the volumes were equal. The scanning technique was the same as we used. A comparison between renal length measured by ultrasound and age enabled a regression equation to be calculated, but there were only 10 infants whose mean renal length was roughly equal to the biggest babies described here.[4] More recently, renal measurements by ultrasound were made in 52 premature infants (all normal size for age) less than 72 hours old; the scanning technique was not described.[5] No difference in measurement between right and left kidneys was found and correlation between kidney length and birth weight was good—better than with body length or body surface area. A regression line with 95% confidence intervals was plotted. This series was, however, small, and the range of birth weight 530 to 3680 g was divided into 25 groups. The errors inherent in such a small number might have obscured the difference in length between left and right kidneys.

The measurement of renal dimension in newborn infants is a practical undertaking, and the measurement inconsistency that occurred in this series was low. Because it was necessary to obtain replicate measurements at the same examination, however, the estimate may be slightly too low. Our data suggest that kidney depth is the dependent variable that can most accurately be measured, probably because the ultrasound image of the kidney outline is less curvilinear in this dimension than in others.

The inconsistency in kidney length measurement was also low. Kidney area measurement is not recommended because the ultrasound scanner calculated this dimension by producing an ellipse that gave the best fit to kidney outline, a technique which is not common to all machines.

The data show a consistent significant difference in shape between the two kidneys; as the babies became larger the left kidney became comparatively longer and thinner, and the right shorter and fatter. It seems likely that the changing shape was caused by the liver, which in the newborn is larger in proportion to the volume of the abdominal cavity than in later life. The right kidney would be compressed longitudinally and expand horizontally thus retaining roughly the same cross sectional area.

There was a significant difference between the sexes in kidney size, boys have larger kidneys than girls in every dimension. The difference was apparent when correlated with both birth weight and head circumference, suggesting that the male fetus has a slightly higher growth velocity than the female and predicting the sex difference in postnatal growth velocity.

The data enable reliable centile charts to be constructed for all three dependent variables by birth weight and head circumference.

Which of the six variables available for correlation are used in the assessment of kidney size in newborn infants will eventually depend on the experience of the operator and the sophistication of the equipment. There is one possible error concerning the use of head circumference: in the rare syndromes associated with atypical head shape (for example, oxycephaly) or more importantly when there has been serious intrauterine growth retardation producing a baby who is 'light for dates' and in whom there is a tendency for 'head sparing', head circumference may be disproportionately large. Kidney centiles in these babies would be smaller when compared with head circumference than birth weight, and it would be necessary to take account of both independent variables to obtain a reliable assessment of renal size. In the current series there were only 10 babies who had a gestational age beyond 38 weeks and birth weight below 2500 g, and none of these had a head circumference of more than 35·2 cm, the mean being 33·2 cm. A large discrepancy between expected and actual kidney size in a low birthweight baby would, with other evidence, suggest that there had been intrauterine growth retardation.

CONCLUSION

It is possible to measure renal size in newborn infants by a standard technique. For increased accuracy a mean of two measurements of length, depth, and area of each kidney should be calculated. A reliable assessment may be obtained by correlating kidney depth or length with birth weight or head circumference, though the latter should be used with caution if intrauterine growth retardation is suspected.

We thank the Newcastle Health Authority Research Committee for financing this project; Mr M Flaherty and Mr S Hall for writing the raw data filing and sorting programme while they were sixth form computer studies students at Ponteland High School, Newcastle upon Tyne; Mr G J Flanagan, department of medical physics, University of Newcastle upon Tyne, for transferring the data; the staff members and nurses of the departments of child health and obstetrics, Princess Mary Maternity Hospital, Newcastle upon Tyne, for their cooperation, and finally Professor M J R Healy for making the software available for fitting the centiles.

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Rh haemolytic disease: continuing problem of management

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Abstract

Eighteen patients with severe Rh haemolytic disease, all of whom underwent fetal blood sampling and intrauterine transfusion, were studied. Twelve babies survived (67%) all of whom were delivered by lower segment caesarean section. There were three intrauterine deaths resulting in late abortion, one stillbirth with trisomy 21, and two neonatal deaths (both from severe prematurity). The traditional prognostic indicators were all inaccurate, and fetal blood sampling and measurement of the fetal packed cell volume were the most direct methods of assessing haemolysis. The progression of severe Rh disease is unpredictable, and we believe that all cases should be referred to specialist centres for advice or treatment.

Severe Rh haemolytic disease has become rare since the introduction of anti-D prophylaxis in the late 1960s.[1] New cases still occur, however, because of failure to administer anti-D, because a 'silent' immunising event occurs, or because the immunising event overwhelms the effect of the anti-D prophylaxis.[2]

Several authors have outlined the current approach to the management of Rh disease, including the use of intravascular transfusion.[3–5] Numbers are necessarily small and the reports of individual groups provide useful information. We report our experience from a single regional unit that receives referrals from Scotland and the north west of England.

Patients and methods

Eighteen patients with fetuses severely affected by Rh haemolytic disease were treated from 1986 until the end of July 1988. They had a total of 50 previous pregnancies, five of which had ended before 28 weeks' gestation because of Rh disease; 45 were delivered after 28 weeks of which eight were stillbirths and one died in the neonatal period (20%).

Indications for treatment during their current pregnancies were: (i) an obstetric history of a previously affected baby with a cord haemoglobin concentration of less than 100 g/l, a baby requiring exchange or top up transfusions, a fetus requiring intrauterine transfusion, or a previous pregnancy resulting in a late abortion, stillbirth or neonatal death from Rh disease; (ii) a change in the optical density 450 of the amniotic fluid, indicating the need for intervention using 'Whitfield's action line'[6]; and (iii) ultrasonographic evidence of hydrops—for example, ascites, skin oedema, and pericardial or pleural effusions.

Fetal blood samples were all taken with a 20 gauge needle and a needle guide attached to a 5 mHz sector transducer (Diasonics, DRF 400). Samples were immediately checked to make sure that they were fetal in origin, and the packed cell volume was used to calculate the amount of donor blood required to raise the fetal packed cell volume to 0·40.[7] Coombs's test, karyotype, blood grouping, infection screen, and biochemical analysis were also carried out on this first sample.

Patients were given oral lorazepam 4 mg and cephradine 500 mg two hours before the procedure, intramuscular papaveretum 10 mg and perphenazine 5 mg one hour before the procedure for pain relief, prophylaxis of infection, and to reduce fetal activity. Diuretics, inotropic or chronotropic drugs, and muscle relaxants were not used. No patient underwent plasmapheresis,[8] and by the time of referral all had raised antibody concentrations (mean=40 IU/ml, range=11–256 IU/ml). The initial fetal blood samplings were carried out at a mean gestational age of 25 weeks (range=18–31), the mean packed cell volume being 0·20 (range 0·08–0·32).

They underwent a total of 72 transfusions: intravascular alone (n=39), intraperitoneal alone (n=14), intravascular (n=8) with additional elective intraperitoneal transfusions (n=8), which were counted as separate procedures. Three intracardiac transfusions were done because of technical problems with cordocentesis. The mean number of transfusions per patient was four (range 0–9). We failed to cannulate the cord 19 times, nine times because the fetus moved.

The data have been divided into groups based on the baby's condition at delivery in the last pregnancy, as this was thought to be the most important prognostic indicator: mild—Coombs's test positive, baby possibly needing phototherapy but no transfusion; moderate—exchange or top up transfusion needed after delivery, and severe—previous late abortion, stillbirths, or neonatal death, cord haemoglobin <100 g/l at delivery or previous intrauterine transfusion.

Results

The timing of the 'sensitising event', the Liley zone of the first sample of amniotic fluid taken, the change in antibody content, the ultrasound appearances at presentation, the packed cell volume at the time of first fetal blood sampling, and the mean gestational age at

Table 1 Predicted severity of Rh disease based on outcome of previous pregnancies and investigation and outcome of present pregnancy

	Mild (n=6)	*Moderate (n=3)*	*Severe (n=9)*
Time of sensitising event:			
Unknown	0	0	1
Silent, antenatal	2	1	5
Despite anti-D	2	1	2
Anti-D not given	2	1	1
Optical density (Liley zone) of amniotic fluid:			
IIB	0	2	1
IIA	4	0	2
IB	0	0	1
Amniocentesis not done	2	1	5
Serum anti-D concentration (IU/ml):			
At time of booking	8	26	53
Mean value by time of first fetal blood sample	40	24	33
Maximum increase	×25	×4	×7
Single:multiple antibody ratio	2:4	0:3	2:7
Appearance on ultrasound scan:			
Normal	3	0	5
Ascites alone	0	2	2
'Hydrops'	3	1	2
Packed cell volume at time of first fetal blood sample:			
>0·30	1	0	1
0·20 to 0·30	3	1	7
<0·20	2	2	1
Mean gestational age at time of first fetal blood sample (weeks)	25	27	24
Mean No of transfusions	3·6	2·8	5·2
Outcome:			
Normal	5	1	6
Abortion	0	1	2
Stillbirth	0	0	1
Early neonatal death	0	1	0
Late neonatal death	1	0	0

the time that the first sample was taken were arranged in the three prognostic groups. There were six patients in the mild group, three in the moderate group, and nine in the severe group (table 1).

SENSITISING EVENT

Only one of the 18 patients had an inadequate explanation for sensitisation (seven had silent antenatal events, and five had overwhelming sensitising events). Silent antenatal sensitisation was more common in the severe group but there were no other important correlations. Anti-D prophylaxis had not been given to five patients.

AMNIOTIC FLUID

Of the 10 patients in whom an initial amniocentesis was carried out, one had optical density 450 in Liley zone IB, six in zone IIA, and three in zone IIB. There were no patients with measurements in zone IA or III.

There was no correlation between the results of amniocentesis at presentation and the prognostic group; eight patients did not have amniocentesis.

ANTIBODY CONCENTRATION AT THE TIME OF BOOKING

The mean antibody concentration in the blood at the time of booking varied with the severity of the past obstetric history, and patients in the severe group were more likely to have higher mean antibody concentrations in early pregnancy than those in the mild group. By the time of the first fetal blood sample, however, the difference between the mild and severe groups had disappeared. There was a noticeable difference between groups when the 'increase to maximum antibody concentration' was considered. There

Table 2 Details of birth, neonatal requirements, and outcome related to severity of Rh disease predicted by obstetric history

Case No	*No of intrauterine transfusions*	*Gestational age at delivery (weeks)*	*Mode of delivery*	*Birth weight (g)*	*Apgar score*		*Cord blood concentrations*	
					1 min	*5 mins*	*Haemoglobin (g/l)*	*Bilirubin (µmol/l)*
Mild disease								
1	3	34	Caesarean section	1940	6	8	122	95
2	7	29	Caesarean section*	1520	1	4†	120	79
3	5	33	Caesarean section	1790	7	7	120	30
4	7	32	Caesarean section	1790	7	8	115	30
5	3	33	Caesarean section	2350	9	9	137	50
6	3	33	Caesarean section	1190	9	9	128	70
Moderate disease								
7	0	32	Caesarean section*	1780	6	9	50	120
8	1	27	Vaginal	1260	Not recorded		48	Not applicable
9	5	27	Caesarean section*	1250	1	3	100	Not applicable
Severe disease								
10	3	32	Caesarean section	2060	9	10	110	100
11	9	33	Caesarean section	2230	9	10	73	144
12	6	32	Caesarean section	2720	7	9	161	89
13	4	32	Vaginal	3060	0	0	Not applicable	
14	6	31	Caesarean section*	2040	8	9	115	100
15	3	22	Vaginal	630	Not recorded		12	Not applicable
16	4	33	Caesarean section	1900	9	10	95	Not recorded
17	2	33	Caesarean section	2700	6	8	146	121
18	2	25	Vaginal	1150	Not recorded		60	Not applicable

*Emergency operation; †Apgar score at 10 mins=7.

was a 25 fold increase in the mild group compared with a sevenfold increase in the severe group. Patients in the moderate and severe groups were more likely to have multiple and rare antibodies.

APPEARANCES ON ULTRASONOGRAPHY

Ten of the 18 fetuses had ultrasonographic evidence of ascites, but of these, six had definite evidence of hydrops (defined as scalp oedema and ascites); one of the fetuses developed these signs during treatment. Patients in the mild group were more likely to have a normal ultrasound scan at the time of fetal blood sampling.

INITIAL PACKED CELL VOLUME

In 16 of the 18 cases the packed cell volume of the fetus was less than 0·3, and there were no differences among the groups. The mean gestational age at which the first fetal blood sample had to be taken and the number of transfusions necessary to treat the fetus adequately were similar in the three groups, except that case 7 in the moderate group did not have an intrauterine transfusion (table 2).

OUTCOME OF PREGNANCY

Twelve babies survived (67%) (table 2). All the babies who had a normal outcome were delivered by elective lower segment caesarean section. Half the babies who were delivered by emergency caesarean section died including two neonatal deaths of babies who were delivered because of acute complications during intrauterine transfusions. There were three intrauterine deaths (chromosomally normal, late abortions), occurring because of sudden deterioration together with technical difficulties during transfusion. The stillborn fetus had trisomy 21 that was detected at the time of the first fetal blood sample (case 13), and intrauterine treatment was abandoned after full discussion with the parents.

NEONATAL OUTCOME

There was no significant difference in birth weight, Apgar scores, cord haemoglobin, serum bilirubin concentration, peak serum bilirubin concentration, number of days of artificial ventilation, number of days receiving oxygen, the need for exchange or top up transfusions, and grouping according to obstetric history. Six of the 10 fetuses with ascites alone or evidence of hydrops at the time of the first fetal blood sample had a normal outcome. The hydrops resolved, after intrauterine transfusion in all cases, apart from the chromosomally abnormal fetus. Five patients of the eight who had normal ultrasonographic appearances at the time of the first fetal blood sample had normal outcomes.

Discussion

Severe Rh haemolytic disease requiring treatment is now uncommon (occurring in roughly one in 6000 pregnancies) and outcome should be optimal if supervision is restricted to a few specialist centres. Not only is the problem rare but the traditional methods of assessing prognosis are inaccurate; there seems to be little correlation between obstetric history and any of the previously used predictors of outcome (table 1).

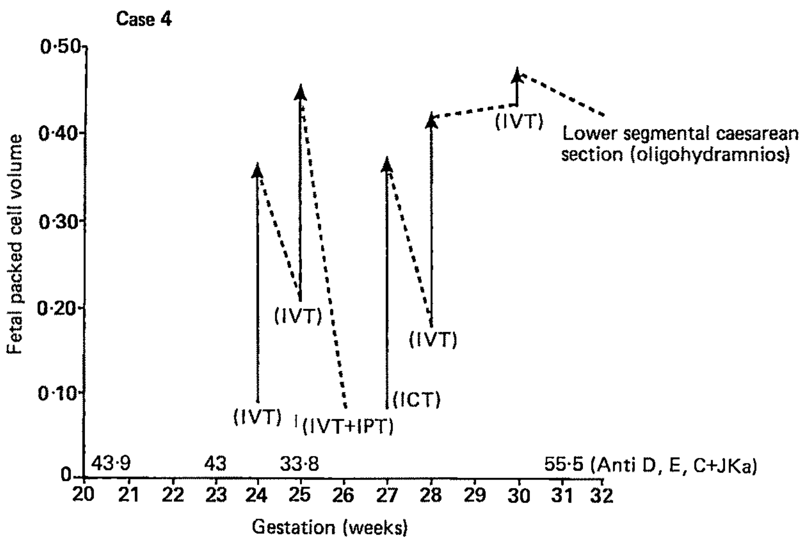
PAST HISTORY

Rh haemolytic disease increases in severity with each subsequent pregnancy. This effect can, however, be unexpectedly aggressive, so that even patients predicted to be in the mild category can have pregnancies affected by devastating disease.

MATERNAL ANTIBODIES

Both the serum antibody concentration at the time of booking, and its change early in pregnancy, are poor predictors of fetal outcome. The rise in antibody content was not always associated with a rapid fall in fetal haemoglobin concentration as might be expected during haemolysis. Indeed, a rise in antibody concentration occurred after the fall in fetal haemoglobin concentration in five cases. The rise in antibody concentration has been considered, at

Peak serum bilirubin concentration (µmol/l)	*No of exchange transfusions*	*No of top up transfusions*	*No of days artificially ventilated*	*Respiratory distress syndrome*	*Cranial ultrasound scan*	*Outcome*
339	4	0	6	Yes	Normal	Alive
280	2	0	11	Yes	Intraventricular haemorrhage	Late neonatal death
Not recorded	0	0	Not recorded	No	Normal	Alive
61	1	1	0	No	Normal	Alive
150	0	1	0	No	Normal	Alive
130	0	1	0	No	Normal	Alive
320	3	0	4	No	Normal	Alive
						Late abortion
						Early neonatal death
320	2	0	0	No	Normal	Alive
219	3	0	1	No	Normal	Alive
245	3	0	0	No	Normal	Alive
						Stillbirth
Not recorded	0	0	0	No	Normal	Alive
						Late abortion
Not recorded	2	0	0	No	Normal	Alive
157	0	1	0	No	Normal	Alive
						Late abortion



Change in fetal packed cell volume and gestational age in case 4. IVT=intravenous transfusion, IPT=intraperitoneal transfusion, and ICT=intracardiac transfusion.

least in part, to be related to cordocentesis itself,[9] but we could find no evidence to support this view and think that it is more likely to be a reflection of the disease.

The antibody concentration, and its rise, have been used in the past to judge the extent of Rh disease, but serious disease may be present in patients with comparatively low antibody concentrations. An increasing proportion of patients with severe disease, however, have multiple and rarer antibodies. More information is required about the transfer of maternal antibodies, antibody avidity, and the fetal factors concerned in acute haemolysis before we can understand the importance of these observations.

AMNIOTIC FLUID OPTICAL DENSITY 450

All our patients showed signs of severe Rh disease quite early in pregnancy, and consequently most of the values obtained indicated the need for urgent intervention. Amniotic fluid analysis has a part to play in mild to moderate disease,[10] but probably not when events dictate intervention before 24 weeks' gestation at which time repeated fetal blood sampling may be a more appropriate investigation.

ULTRASONOGRAPHY

This seems to be of limited value in the assessment of Rh disease,[11] as deterioration may be so rapid that an apparently normal fetus one day may have become hydropic the next. It did prove useful, however, in monitoring treatment and the resolution of hydropic features after transfusion.

FETAL BLOOD SAMPLING

Direct measurement of the packed cell volume of the fetus gives precise information about the progress of the disease and permits accurate calculation of the volume of blood required for transfusion. Other 'causes' of fetal hydrops may also be excluded, and the fetal karyotype assessed.

The figure shows the changes in the fetal packed cell volume with repeated intrauterine transfusions in one patient, and shows the typical peaks after transfusion and troughs beforehand. As treatment proceeds, fetal red cells disappear as a result of destruction and marrow suppression. The packed cell volume stabilises, so that the interval between treatments may be lengthened; these may be further extended by combining intraperitoneal and intravenous transfusion.

SENSITISING EVENTS

A good case can be made for the introduction of routine antenatal prophylaxis with anti-D, which appears to reduce the incidence of 'silent' antenatal sensitisation; seven of the 17 patients in whom the method of sensitisation was known became sensitised in this way. Of concern is the large proportion of patients who never received any anti-D at the time of a sensitising event or at delivery.

CONCLUSION

Rh haemolytic disease is still a serious problem for those few affected pregnancies and it is unlikely to disappear. Although it is a rare condition it necessitates intensive management from both obstetric and paediatric units. All potentially affected cases, possibly with the exception of patients with only traces of antibodies, should be referred as early as possible to a regional unit for either advice or treatment. This need is emphasised by the rather unpredictable course that Rh disease now seems to follow.

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Decline in breast feeding

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Abstract

Information on whether mothers intended to breast feed and whether they actually did so one month after delivery has been recorded for all mothers in Sheffield during the last 15 years, and changes in the last 10 years have been reported. There was a steady increase in the percentage of mothers intending to breast feed and in those who did so in the 1970s. From 1984–8, however, there has been a rapid and progressive decline, despite an increase in mean maternal age and a reduction in the number of non-white births. The decline in the percentage of mothers who intend to breast feed has been greatest among those whose education did not extend beyond the age of 18, and among Asian mothers.

During the 1970s there was much interest in breast feeding and the salt content of milk fed to babies. During the period 1974 to 1977 in Sheffield there was a progressive increase in both the intention and the achievement of full breast feeding,[1] but by 1977 there seemed to be some levelling off. In 1988 concern was expressed in the *Lancet* about the failure of progress in breast feeding that had been reported by the DHSS the same year.[2 3] The breast feeding rate in this country had been static during the previous five years.

Recently we resurveyed the breast feeding pattern in Sheffield and were disturbed to find that since 1981 there has been a rapid and continuous decline both in the intention and achievement of breast feeding, and in this paper we record both this observation and our attempt to identify the groups concerned and the factors that may have been influential.

Patients and methods

As part of the Sheffield Child Development Study, information is obtained on every baby born to residents of Sheffield. This includes the age of the mother, her intention to breast or bottle feed, the age at which she completed full time education, her previous pregnancies and their outcome, and her ethnic group. One month over delivery the health visitors do a home visit at which they collect data that includes the baby's feeding regimen. There are roughly 6000 births a year, and this study comprises birth and one month data on 55 100 mothers, over 98% of all mothers delivered during the period July 1979 to May 1988.

Results

Sheffield has three main maternity units in which over 98% of all babies are delivered. One unit (Nether Edge Hospital) is on the west, close to the more affluent areas of the city; the second unit (the Northern General Hospital) on the opposite side of the city serves the inner city areas and the east and, in the centre of the city, is the Jessop Hospital for Women within the university campus, which contains the main neonatal intensive care unit.

As these hospitals serve different populations, the breast feeding achievements have always been different. This can give insight into what is happening in the city, and therefore—in addition to the overall figures—we present the data from the three units separated. The proportion of births in the three units has changed during the 10 years of the study; births at the Nether Edge Hospital have decreased from 30% to 25%, those at the Jessop Hospital for Women have increased from 31% to 37%, and those at the Northern General Hospital decreased from 37% to 35%.

The trends in intention to breast feed and achievement of full breast feeding for the whole city for the period 1979–88 are shown in fig 1. The figures in all the illustrations are presented in percentages, the number of observations in

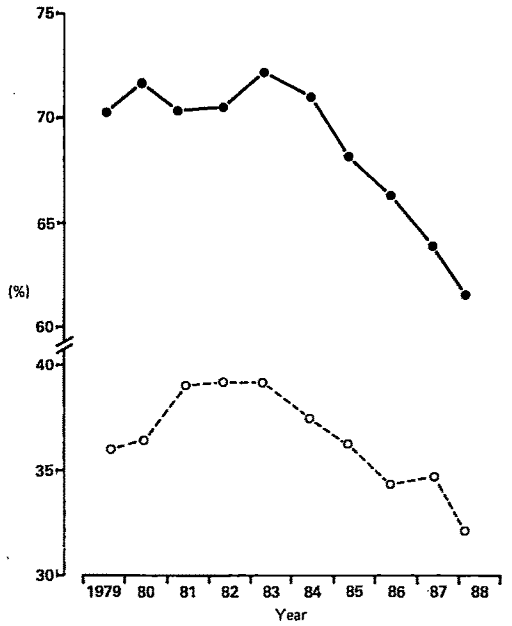

Figure 1. Percentage of mothers who intended to breast feed (upper graph) compared with percentage who were actually doing so at one month (lower graph), 1979–88.

each year group being about 6000. The intention to breast feed in 1981 was a little over 70%, and the achievement at one month nearly 40%. By 1988 the intention to breast feed had dropped by roughly 10%, and achievement from 39% to 32%, which means that about 400 fewer mothers were breast feeding their babies at one month in 1988 than in 1982.

There was a reduction in intention to breast feed in all three units, but the greatest reduction occurred in the Jessop Hospital for Women (fig 2). Achievement produced a different pattern. In the unit in the more privileged part of the town (Nether Edge Hospital) achievement remained static, but fell in the academic unit (Jessop Hospital for Women) at a greater rate than in the less privileged Northern General Hospital (fig 3). Factors which might have influenced this decline were investigated.

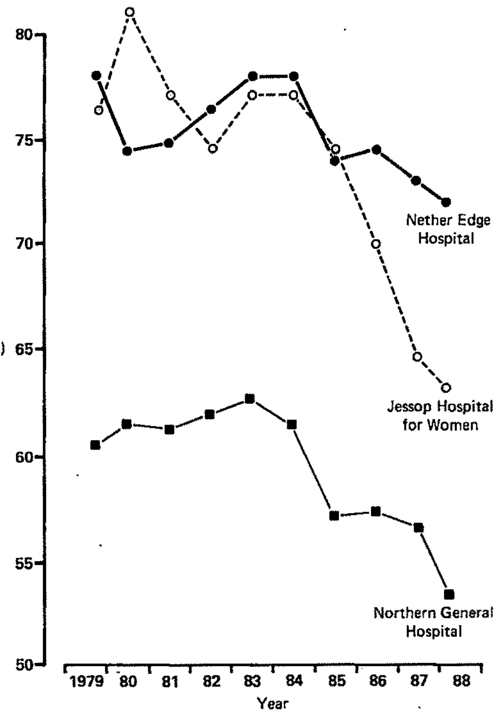

Figure 2 Percentage of mothers who intended to breast feed, and hospitals at which their infants were delivered, 1979–88.

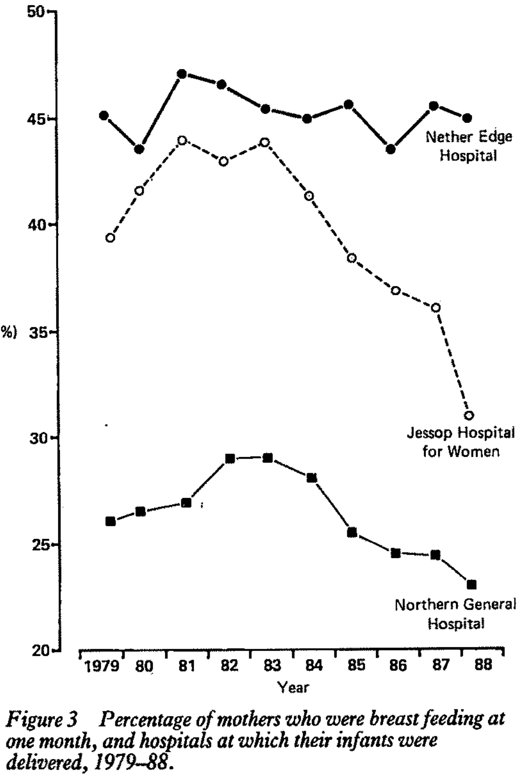

Figure 3 Percentage of mothers who were breast feeding at one month, and hospitals at which their infants were delivered, 1979–88.

PARITY

During the past eight years there had been no appreciable local trend in parity. There has, however, been a rapid and parallel reduction in intention to breast feed, whatever the mother's parity, since 1984. The comparatively higher rate of intention to breast feed first babies has continued, but achievement rates are similar for first, second, and third children. Mothers with more than three children have maintained their previous rates of achievement.

MOTHERS' AGE

The average age of mothers increased slightly but steadily throughout the period. The number of mothers under the age of 20 remained almost constant at around 10%. Those aged between 20 and 25 fell from 33% to 28%, and those over 25 rose from 55% to 62%. There has been a consistent association between mother's age and both intention to breast feed and achievement, the older the mother the greater both intention and achievement. The fall in intention after 1983 was the same in all age groups. The percentages of mothers achieving full breast feeding have shown a tendency to diverge, the percentage of mothers under the age of 20 being below 15%. That in mothers aged 20 to 29 is 30%, and in those over 30 around 45%.

EDUCATIONAL LEVEL OF PARENTS

The intention to breast feed among women whose full time education continued after their 18th birthday has remained almost static at around 90%, whereas the intention to breast feed in women completing their education before their 18th birthday was static at about 68% from 1979–83, and then declined to about 58% in 1988.

ETHNIC GROUP

The only non-white group big enough to show useful trends was the Asian group. The intention to breast feed among both Asian and white mothers shows a pronounced fall since 1984, the Asians having their highest peak in that year, a year when the intention levels of the two groups were not significantly different (fig 4). The degrees of achievement of both Asian and white mothers decreased, among Asian more than among white mothers.

WEIGHTS OF BABIES

The mean weight of babies born during this period has remained static. There was an initial increase in the proportion of babies weighing

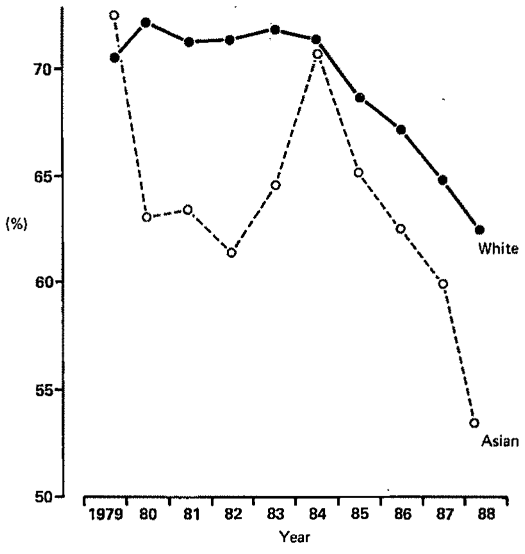

Figure 4 Percentage of white and Asian mothers who intended to breast feed, 1979–88.

less than 2000 g, but this stabilised after 1983. When we related breast feeding intention and achievement to birth weight we found a clear correlation between birth weight and both intention and achievement. Among babies weighing less than 1000 g the achievement was nil; among those between 1000 and 2000 g, 13%; among those between 2000 and 3000 g, 29%; and among heavier children, about 37%. The intention rate showed a similar correlation.

LENGTH OF STAY IN HOSPITAL

The mean length of mother's stay in hospital has been getting progressively less over the last 10 years in all three units. Though the intention to breast feed in full and short stay mothers has decreased in all groups, the level of intention was similar. The achievement of both groups fell, but the achievement of the long stay mothers was consistently lower than that of the short stay mothers.

Discussion

The breast feeding rates in this country fell from around 50% in 1930 to 10–20% in the early 1970s, and then rose rapidly in the later 1970s.[4] There was a similar pattern in the United States with a similar levelling out about 1982.[5 6]

Sheffield experienced a steady rise in the proportion of mothers successfully breast feeding during the 1970s,[1 7] but the years 1981–7 have shown a progressive and rapid decline in both the number of mothers intending to breast feed and the number fully breast feeding at one month. This decline has taken place during a period of time when factors such as increasing maternal age and birth weight (which are known to enhance breast feeding rates) have either increased or remained static.

Many studies have shown a strong correlation between social class and both intention and success in breast feeding.[5 7 8] We did not record social class, but the age at which the mother completed full time education probably reflects this. The discrepancy between the success rates from the three different maternity units during the earlier study[1] could be entirely explained by this single factor.[9] In the present study the effect of continuing education remains pronounced, with mothers whose education continued after their 18th birthday being more than twice as likely to breast feed as those completing their education earlier, and the decline in breast feeding over the study period was almost completely confined to the latter group (fig 5). It is notable that the one unit with a high proportion of mothers who had had further education was the only unit in which there was not a decline in breast feeding achievement.

A possibility is that the decline in intention and achievement in the Jessop Hospital for Women (which houses the regional neonatal intensive care unit), could be the result of the concentration of births of small babies and mothers with obstetric problems in this unit. The total number of babies weighing under 2000 g born in 1983 was 143; in 1984, 140; in 1985, 163; in 1986, 170; and in 1987, 151. In 1987 there were roughly 230 fewer mothers achieving breast feeding from the Jessop Hospital unit than there were in 1983. Thus if every low birthweight baby in the city was born at the Jessop Hospital, the numbers could not account for the overall pattern of change in that unit. It is possible that the recent emphasis on the disadvantages of breast feeding for specific groups such as very low birthweight babies, or the babies of HIV positive mothers,[10] has influenced the attitudes of staff towards breast feeding in units in which such babies are concentrated. Conversely, the unit with greatest success in maintaining breast feeding (Nether Edge Hospital) is the one concerned principally with normal mothers and babies.

Social factors that may affect rates of breast feeding are the proportion of mothers returning to work[11] or a reduction in the availability of maternity leave.[12 13] In Sheffield the recent

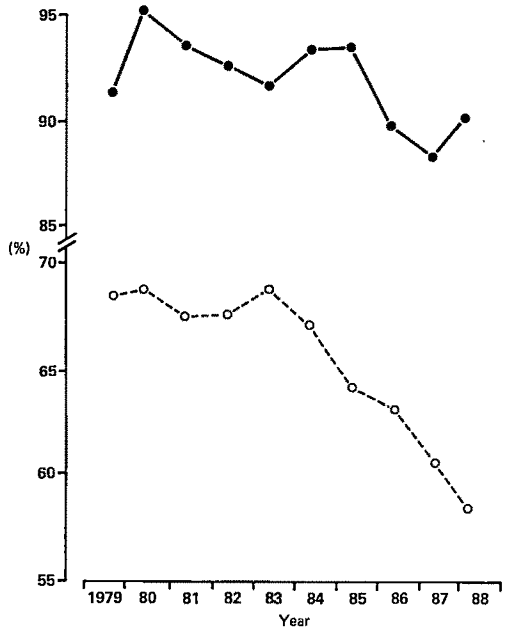

Figure 5 Percentage of mothers who intended to breast feed divided according to the age at which they finished full time education: 18 years and over (upper graph) and under 18 years (lower graph), 1979–88.

decline in breast feeding has taken place during a period of high unemployment and these factors are not likely to have been important.

The lower rate of intention and achievement in Asian mothers could be explained by an influx of Asian mothers into the city. The proportion of Asian mothers varied between 5·3% and 6·2%, with a tendency to decline towards the end of this period, which should cause the trend to be in the opposite direction from that observed.

Most studies of intention to, and success in, breast feeding are of mothers and babies attending a single maternity or paediatric unit. The study reported here is a 'population study', information being recorded about all babies born to parents resident within the city boundaries during the period of the study. We therefore feel that we have identified a true population trend.

Earlier studies have shown that the decision to breast or bottle feed is usually taken before conception,[14] and that family rather than professional influences are paramount. The discrepancy between intention and success, however, must at least be partly because professionals are unable to give adequate time to the active promotion and encouragement of breast feeding. Pressure on community based services to place their priorities elsewhere and the trend promoted by the government towards larger lists for family doctors must mean that family doctors, midwives, and health visitors will have less time to spend on this aspect of primary preventative paediatrics, and there is evidence that—at least in some countries—promotion by manufacturers of milk products is increasing.[15]

We have not been able to identify a single cause for the recent decline in breast feeding in Sheffield. The biggest decline in intention and achievement has been among Asian mothers and among mothers who did not receive further education. The decline has taken place over a period of time when the city itself was under stress from unemployment, during which the health care services have been increasingly stretched, and when postperinatal mortality stopped decreasing.[16]

We thank the midwives and health visitors of Sheffield for their continuing recording of information for the Sheffield Child Development Study, and the 'Children's Hospital Research In Sheffield' fund and the Foundation for the Study of Infant Deaths for financial support.

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Pulsatile weight increases in very low birthweight babies appropriate for gestational age

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Abstract

The weight increment profiles of 20 low birthweight babies measured during the first two months of extrauterine life were analysed. The babies were weighed daily, and the weight profiles showed minor irregularities when compared with an interpolated linear trend. When increments were plotted at two week intervals a linear increase in weight velocity was seen, but when increments were computed every three days, the velocity profile was non-linear and pulsatile. All cases studied showed regular pulsatile patterns of weight velocity during the first two months of life. A mean profile of the 20 babies permitted estimation of the periodicity of the pulsing: the cycle alternated every nine to 11 days. A non-linear pattern was found in the published series of unsmoothed data that have been widely adopted as standards for growth in low birthweight babies.

Survival of low birthweight infants is strictly related to the birth weight and to the weight increments after birth. The aim of this paper was to evaluate the adequacy of the growth centile charts in common use, most of which were derived from cross sectional data.[1–7]

Many of the available centile charts were constructed by pooling data from different centres, as low birthweight babies are comparatively rare. The actual drawing of the centile lines through the mean values (and the points derived by mean (SD) units) were calculated by graphic,[3] as well as mathematical smoothing (three point means, for example, in the charts of Lubchenco *et al*[4]). Most data points are derived by linear extrapolation from raw data points, with an assumption of linearity between one time and the next; regression methods were often used.[1 3 7]

Few longitudinal studies have been carried out, and data from individual subjects are rare, as all published reports deal with pools or group means. The velocity, or the dynamic growth of the individual low birthweight infant is a good monitor of his well being, and is carefully monitored in each nursery, but is rarely published. A velocity curve may be helpful to monitor the adequacy of the increments of the individual subject. The object of this paper was to analyse the individual patterns of weight increase of a small cohort of low birthweight babies who were weighed accurately every day. Length measurements were not considered because of the potential for errors of measurement.

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Subjects and methods

Twenty low birthweight babies were weighed nude before feeding every day on a Vandoni scale accurate to the nearest 5 g by experienced nurses. The mean (SD) difference in measurements (obtained by 64 duplicate measurements) was 12·2 (3·4) g.

Eleven girls and nine boys were born between 26 and 30 weeks' gestation with birth weights ranging from 930 to 2000 g. Most had the common complaints of preterm babies: six required assisted ventilation, 15 were given blood transfusions, seven had apnoeic episodes, five had severe jaundice, and 11 had sepsis.

Velocities were expressed in g/day, and were calculated by dividing the increments between two intervals by the days, and plotting the mid interval between measurements.

Results

Figure 1 shows the weight profile of a baby born

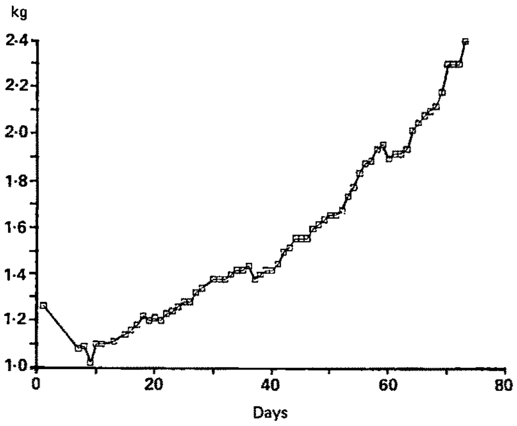

Figure 1 Weight profile of case 1.

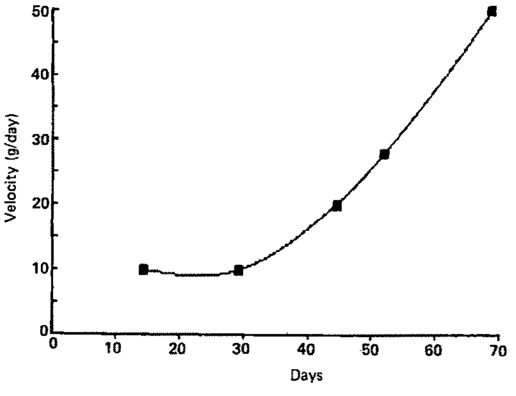

Figure 2 Weight velocity profile obtained by computing increments at 15 day intervals (case 1).

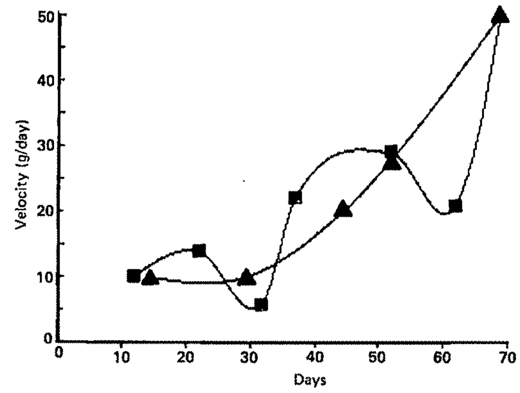

Figure 3 Weight velocity profile obtained by computing increments at 15 and 10 day intervals (case 1). The triangles indicate 15 day measurements and the squares 10 day measurements.

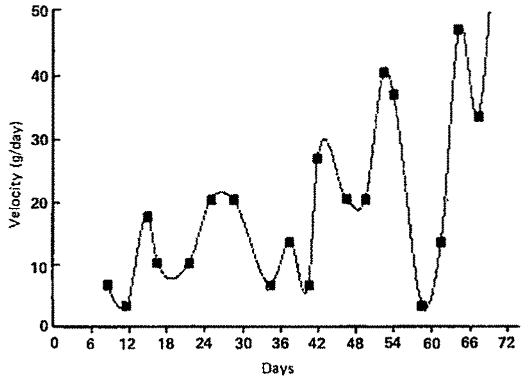

Figure 4 Weight velocity profile obtained by computing increments at three day intervals (case 1).

at 29 weeks' gestation weighing 1260 g: daily data points were plotted. Figure 2 shows the velocity obtained by weights taken at 15 day intervals: there is a smooth continuous line, similar to the line of daily weights. When we consider the weight velocity at 10 day intervals (fig 3) a different type of line is obtained, but when velocities were computed using weights at three day intervals a pulsatile velocity pattern was seen (fig 4). The non-linear pattern was present in the raw data (fig 1), but is completely obscured by linear smoothing. The weight velocity of the child goes upwards by a series of spurts and lags. Lags could not be linked to adverse clinical events: though on days 19–20 the child did have an episode of infection, and at days 37, 48, and 60 there were no serious clinical events.

Each of the other 19 velocity plots suggested a pulsatile pattern, which did not seem to vary among subjects. Six unselected cases are shown in fig 5, as examples. The other raw data are available from the authors. From the velocity data we took the time at occurrence of each peak and the velocity at that time point, as well as time and velocity at each nadir following the peak, for each subject. We then computed the mean (SD) of each peak and its subsequent

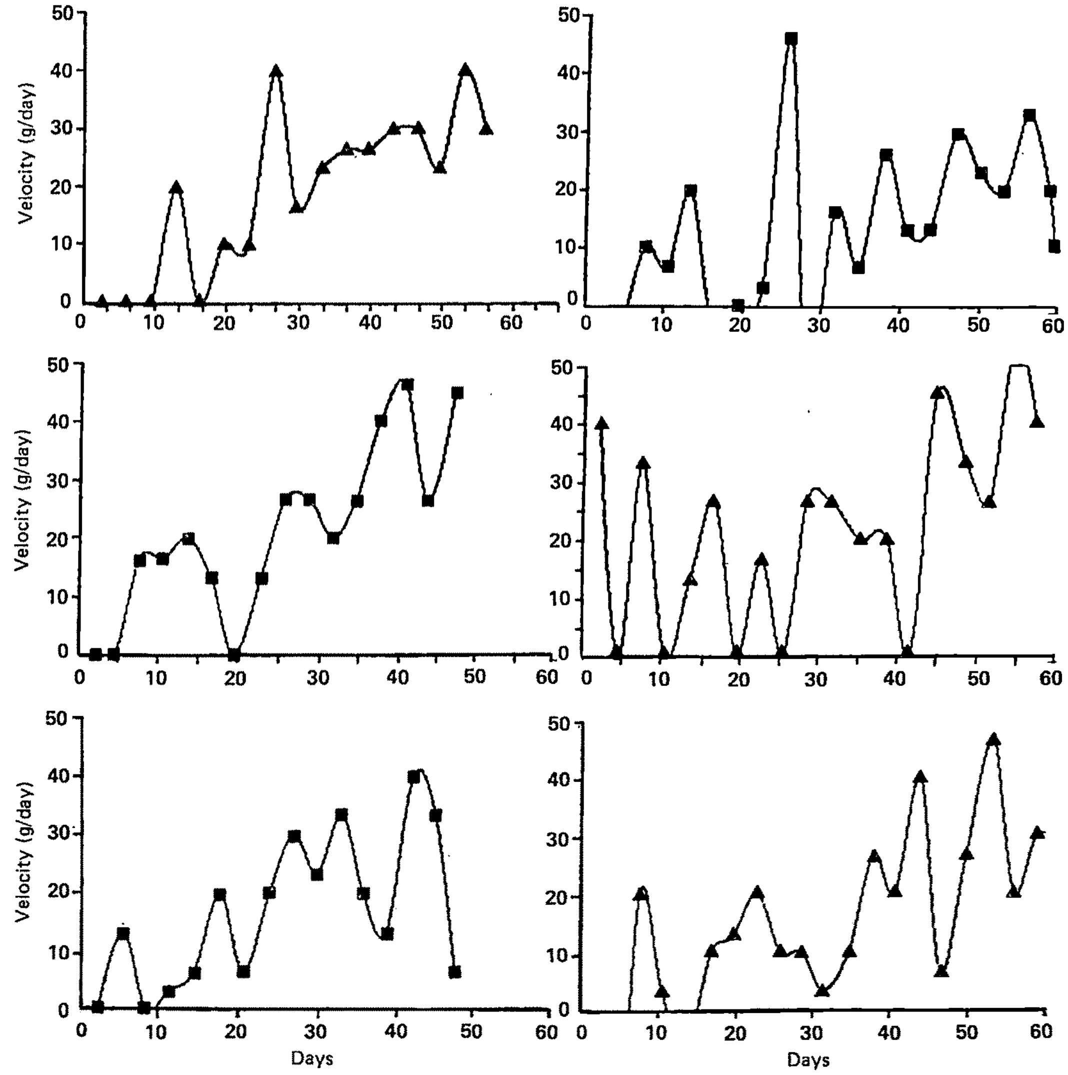

Figure 5 Three day weight velocity profiles of six low birthweight babies.

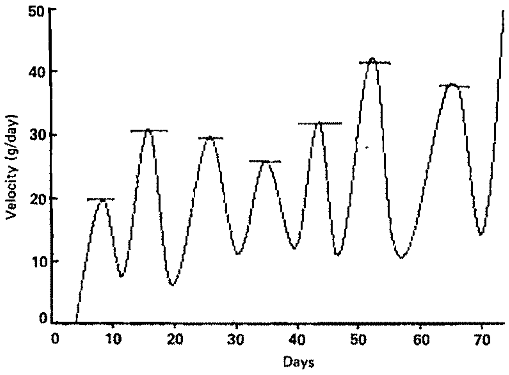

Figure 6 Profile of the mean weight velocity pattern of 20 low birthweight babies. Horizontal lines indicate 1 SD intervals of the peaks.

nadir. No smoothing was applied to the data points and no assumption about an underlying mathematical model was made.

Figure 6 shows the pattern obtained by the plot of these mean values. A constant pulsatile pattern is suggested by the graph, with a distance from peak to peak of eight to 11 days (mean (SD) 9·55 (2·18) days).

Discussion

Low birthweight babies increase their weight by a pulsatile rhythm, the period between peaks being about 10 days. Their weight increase is not linear as previously reported; the linearity is only the result of the extensive use of graphic as well as of mathematical smoothing. The currently available centiles charts are useful in clinical practice, in that they allow comparison of an individual with a group mean, but they may not be adequate for the plot of longitudinal data of a single subject. New studies are therefore required, as well as a re-evaluation of the longitudinal data already available.

The lack of velocity data in the neonatal period may be because of the inaccuracy of the measurements. To get firm velocity data the longest period between two measurements must be taken, as short time growth evaluation may be misinterpreted because of such inaccuracies of measurement. As far as weight increments are concerned, most reports give an error of measurement of about 10 g.[1 3 7] For example, a low birthweight infant will gain an average of 300 g between the 32nd and the 33rd week, which is 30 times larger than the error of measurement.

Velocity data may therefore be interpreted with adequate control of the error of measurement, at least as far as weight is concerned. The general assumption about the growth of low birthweight infants is that it is linear, which is the philosophy underlying the concept of the centile graph in low birthweight babies as well as in those born at full term of normal birth weight.[1 3 4] Specific statements have ruled out the possibility of a growth process that is not continuous and linear.[8] Nevertheless, it is generally accepted that a baby born with a low birth weight before full term has to go through some variation in growth velocity, as he has to go through a period of negative velocity, a period of catch up growth, and a period of stable velocity. Gairdner and Pearson described 'one of the commoner patterns of weight gains to be seen in prematures and small for date infants' in four 'phases': the first of weight loss, the second (after the first week) parallel to a fetal weight curve, the third of growth acceleration and catch up, and the fourth of stability along the individual centile.[2]

Brandt reported accurate individual growth data of a cohort of 64 infants whose weights were appropriate for their gestational age and 43 who were small for their gestational ages, with weekly, fortnightly, and monthly measuring intervals. The velocity curves were drawn from the means of the velocity of each individual, but they still showed some irregularities, possibly because of the multiple peaks that indicated the height as well as the weight values.[7] If we consider the widely used reference data of Lubchenco *et al*,[4] the plot of the velocities obtained by points interpolated on the smoothed 50th centile line give a single peak of maximum velocity around the 34th week of gestation, but if we compute the increments between the pooled mean weights given for boys and girls in the same paper, the pattern is that of a pulsatile growth, although cross sectional pooled data of both sexes with a comparatively wide interval between measurements are considered.

The suggested pulsatile pattern of growth is not unique to the weight of low birthweight babies: most probably it is a universal pattern of the physiology of growth. It may be found in the growth of primates,[8] and in almost every human measurement in which it has been sought. Healthy subjects measured longitudinally over a long period and with great accuracy showed a variation with time, with 'mini spurts' alternating with lag phases during the whole growth period in weight, height, urinary urea concentration, and creatinine excretion.[9 10] The application of high precision knemometry to a wide variety of subjects, both healthy and affected by growth disorders, showed a pulsatile pattern in the growth of the leg in all the subjects studied.[11]

Children with coeliac disease showed a pattern of pulsatile growth in height as well as in weight during the catch up growth that followed the introduction of the gluten free diet; the cycle showed a periodicity of about two months, and during that period the growth velocity was two to four times that expected for the age of the child.[12]

The mathematical models recently developed to fit the human growth curve are adequate for describing the general trend of the growth profile, but new time series models have to be developed to fit the velocity variations of short term growth adequately.

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Tuberous sclerosis presenting with fetal and neonatal cardiac tumours

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Abstract

Cardiac tumours were identified on ultrasonography in fetal or early postnatal life in five infants. Tuberous sclerosis was subsequently diagnosed in all five. Only one infant required operation. Regression of the tumour occurred in three. No infant had hypomelanotic macules at birth, and they took up to two years to appear.

Cardiac tumours are a common feature of tuberous sclerosis, but they are rarely diagnosed in life unless they are causing obstructive symptoms or arrhythmias. Echocardiographic studies, however, have shown tumours in over half of affected patients, none of whom had cardiac symptoms.[1–3]

Cardiac tumours have not been described as the sole sign of tuberous sclerosis in adults,[4] but our experience with fetal and neonatal cardiac tumours shows that they may precede the skin and neuroradiological signs of the disease.

Case reports

CASE 1

A 5 day old baby was referred for a cardiologist's opinion because a murmur had been noted on routine examination. He had no symptoms and his electrocardiogram was normal. Echocardiography showed multiple cardiac tumours, the largest of which was partially obstructing the left ventricular outflow tract. Serial echocardiograms showed spontaneous regression of the tumours so that when last seen (at the age of 5 years) only one tumour 8 mm in diameter remained in the right ventricle. Illustrations of regression of the tumours in this child have previously been reported by Smith *et al* in their figures 2–6.[3]

Careful examination of the skin, and computed tomography scans of the brain, were carried out at 2 and 5 months but failed to show any signs of tuberous sclerosis. By the age of 12 months, however, a streak of depigmented scalp hair and a depigmented macule 3 mm in diameter had appeared. Further depigmented skin patches appeared over the following year. Atypical absence seizures developed at the age of 2 years.

Both parents were investigated by careful skin and fundoscopic examination, computed tomography, radiographs of the hands, and cardiac ultrasonography. No signs of tuberous sclerosis were found. The child's father has Wolff-Parkinson-White syndrome, and the mother has facial acne rosacea.

CASE 2

A healthy 21 year old mother had a normal pregnancy until 34 weeks' gestation, when an irregular fetal bradycardia was noted. Ultrasonography of the fetal heart was normal at 34 weeks, but a further scan a week later showed echodense areas in the right ventricle that persisted until delivery.

During the neonatal period the baby continued to have episodes of bradycardia and multifocal ectopic beats. Cardiac ultrasonography showed three intracardiac masses in the right ventricle. Cardiac catheterisation confirmed the presence of the tumours, and also showed irregularity of the ventricular septum suggestive of additional pea sized tumours within the septum. Over the next two years serial ultrasound scans showed regression of all the tumours to minute proportions.

Cranial ultrasonography at the age of 7 days suggested some increased echodensity in the left frontal region. Renal ultrasonography, and examination of the skin with a Wood's light gave normal results. Computed tomography and magnetic resonance imaging at the age of 7 weeks showed two areas of decreased attenuation in the frontal horns that were similar to the changes of resolving infarction. Despite further examination with the Wood's light, no depigmented patches were found until the age of 2 years when seizures and facial angiofibromas developed, and periventricular calcification was seen on computed tomography.

Both parents were examined for evidence of tuberous sclerosis; there were no cutaneous stigmata, and results of computed tomograms, renal ultrasound scans, and examination of the fundi were normal.

CASE 3

A 22 year old woman with tuberous sclerosis was scanned routinely during her first pregnancy. Fetal cardiac tumours were first identified in the right and left ventricles at 28 weeks' gestation. The pregnancy proceeded uneventfully to normal delivery at 42 weeks. The baby was symptom free at birth, and postnatal echocardiography confirmed the presence of ventricular tumours measuring 10×10 mm on the left, and 10×8 mm on the right. Serial scans showed the tumours becoming progressively less dense during the first 12 months of life.

Examination of the skin in the newborn period failed to show any other signs of tuberous sclerosis. At the age of 11 weeks the baby had a focal seizure. A computed tomogram

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showed a number of calcified periventricular nodules that were characteristic of tuberous sclerosis, but examination of the skin showed no abnormality. Depigmented skin patches did not appear until the age of 14 months, and by 17 months he was developing facial angiofibromas.

The mother underwent echocardiography postnatally and two tumours, each 5 mm in diameter, were found in her left ventricle.

CASE 4

The mother of case 3 had a second pregnancy in 1988. Antenatal scan at 32 weeks' gestation showed multiple small echogenic areas in the lateral wall of the fetal left ventricle, the interventricular septum, and in the right ventricle near the tricuspid valve. The pregnancy proceeded uneventfully to normal delivery at full term.

Postnatal echocardiography confirmed the antenatal findings. Cranial ultrasonography and examination of the skin with a Wood's light showed no abnormality. Mother and baby were discharged after 48 hours, and the baby remained symptom free. At the age of 5 months, two depigmented macules appeared on the chest and left knee. When last seen at the age of 18 months his speech and motor development were normal.

CASE 5

A 17 year old mother underwent a routine scan at 20 weeks' gestation in her first pregnancy. A single large tumour was identified in the fetal right ventricular outflow tract. At birth the baby was well and examination of the skin with a Wood's light, and cranial ultrasonography, failed to show any additional features of tuberous sclerosis. Possible seizures at 4 months prompted computed tomography of the brain, which showed multifocal periventricular calcification. A month later, because of potential outflow tract obstruction, the baby underwent resection of a solitary cardiac tumour, which histological examination showed to be a rhabdomyoma. When last seen at the age of 18 months, examination of the skin showed no abnormality, speech development was delayed, and he had focal and generalised seizures that required treatment with valproate and nitrazepam.

His mother's medical history was reviewed. She had developed papules on her cheeks at the age of 6 years and had consulted a dermatologist, but not been given a diagnosis. She had had bilateral spontaneous pneumothoraces during pregnancy.

On further investigation, multiple echogenic lesions characteristic of angiolipomas were seen on renal ultrasonography, and there were cerebral changes consistent with tuberous sclerosis on her computed tomogram. Examination of the skin with a Woods light did not show any depigmentation.

Discussion

Tuberous sclerosis is an autosomal dominant condition with high penetrance, and an estimated prevalence between 1/15 000 and 1/30 000. There is a slight male preponderance, and 50 to 80% of those affected seem to have resulted from new mutation.[5]

Cardiac rhabdomyomas are among the earliest signs of tuberous sclerosis and occur in over half of affected patients.[1–3] By contrast, primary cardiac tumours are rare in the general population, and extremely uncommon in childhood.[6] Necropsy studies have suggested that 37–50% of those dying of cardiac rhabdomyomas have tuberous sclerosis.[7 8] This is almost certainly an underestimate because the studies include a high proportion of stillborn infants and neonates, who would not necessarily show any other diagnostic features.

In the present series the cardiac ultrasound appearances were characteristic of rhabdomyoma, and all the infants subsequently developed other signs of tuberous sclerosis. Cases 1 and 2 illustrate the difficulty in establishing a firm diagnosis of tuberous sclerosis despite extensive investigation and keeping the possibility in mind. It is probable that most infants with cardiac rhabdomyomas have tuberous sclerosis,[9] and this diagnosis must not be discounted because the initial examination of the skin and computed tomograms give normal or atypical results. The areas of decreased attenuation on computed tomography in case 2 probably represented areas of demyelination rather than infarction, but it is unusual to find these without periventricular nodules.[10] Initial results of magnetic resonance imaging in tuberous sclerosis suggest that this may be a more sensitive method of detecting early changes (M Gomez. Presented at the Tuberous Sclerosis Association Symposium, 1988). Cranial ultrasonography gave abnormal results in one of the three cases scanned in the neonatal period, and its place in the diagnosis of tuberous sclerosis requires further evaluation.

The cardiac tumour detected at 20 weeks' gestation in case 5 is the earliest recorded instance of a fetal tumour associated with tuberous sclerosis. Tumours in the fetuses studied increased in both size and number towards full term, suggesting that maternal factors influence their growth. The only infant with symptoms in this series developed arrhythmias before birth, and before tumours were recognised. Postnatal tumour regression has so far been recorded in three of the five infants, and this also seems to be an active process and not merely the effect of growth of the cardiac chambers.

Despite the usually benign nature of rhabdomyomas, they may cause death from mechanical obstruction or disturbance of rhythm. In the largest necropsy series, 70% of the deaths occurred in the first year and the authors recommended that patients with symptoms should have operations.[9] The availability of ultrasonography permits detection of cardiac tumours in fetal and early neonatal life, and raises questions about appropriate management. There are case reports of successful resection after the prenatal detection of tumours,[11 12] but as tumour regression is now well documented we strongly recommend a conservative approach, reserving operation for those infants with life threatening disturbances of rhythm or outflow tract obstruction by a solitary tumour.[3 13]

Antenatal detection of fetal cardiac tumours should prompt careful examination of parents and siblings for signs of tuberous sclerosis, whether or not this diagnosis is established in the baby. Most authors recommend detailed skin examination with a Wood's light, fundoscopy, renal ultrasonography, and computed tomography of the brain. Even though prevalence of the tumour is low in adults,[3] we include echocardiography, as cardiac tumours are an important marker of the condition. Termination of pregnancy has been carried out after the detection of cardiac tumours in the fetus of an affected mother.[14–16]

Tuberous sclerosis shows pronounced variation in phenotypic expression, and the characteristic skin lesions and computed tomographic signs are often absent at birth. Hypomelanotic macules eventually appear in 90% of those with tuberous sclerosis, and have been regarded as the earliest sign of the condition. Unfortunately the sign is not specific to tuberous sclerosis, as 2–3/1000 normal newborn infants will also have one or two such patches. Late appearance of hypomelanotic macules in tuberous sclerosis is recognised,[17] and in this series none was identified in the neonatal period despite careful examination with a Wood's light. Four of the five children went on to develop hypomelanotic macules, although they took from five months to two years to appear.

We suggest that most fetal or neonatal cardiac tumours are diagnostic of tuberous sclerosis. Multiple ventricular tumours are highly likely to be associated with tuberous sclerosis,[7 9] and the diagnosis should be considered even when a single tumour is detected, as a quarter of the tumours found on ultrasound screening of adults and children with tuberous sclerosis are solitary.[3] As cardiac tumours may remain the sole sign of the condition for many months, families should be offered a presumptive diagnosis and genetic counselling even when other signs of tuberous sclerosis are absent.

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Rectal-skin temperature difference in septicaemic newborn infants

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Abstract

Serial skin (sole) and rectal temperatures were simultaneously taken from 55 healthy and 26 septicaemic newborn infants to find out prospectively whether septicaemic newborn infants have any thermoregulatory reaction to the septicaemia, and whether regular temperature measurements could help in the early diagnosis of septicaemia. The septicaemic infants were divided into three groups: the first comprised eight feverish infants, the second 11 with normal temperatures who were in relatively good clinical condition, and the third seven with normal temperatures who were in poor clinical condition. All 55 healthy babies had rectal temperatures of less than 37·8°C and a mean rectal-sole temperature difference of 2·5°C. The first group of septicaemic infants had rectal temperatures over 37·8°C and a high mean temperature difference of 6·9°C, whereas the second group had rectal temperatures less than 37·8°C and a mean temperature difference of 4·7°C. Infants of the third group had a low rectal temperature and a low mean temperature difference (1·1°C).

We conclude that septicaemic newborn infants show an adequate thermoregulatory reaction, which is reflected by a widening of the rectal-sole temperature difference of more than 3·5°C, except for those who are critically ill, who lose this ability. In view of these results infants with normal temperatures but with a rectal-sole temperature difference of more than 3·5°C should be suspected of septicaemia and investigated thoroughly.

Fever is a common sign of infection in children and adults. The regulation of body temperature at a raised point (fever) is achieved by two main mechanisms: firstly, increase of heat production by shivering or non-shivering thermogenesis and, secondly, decrease of heat dissipation, mainly by peripheral vasoconstriction. The end result of these mechanisms is a widening of the gap between the core (rectal) and skin temperature gradients.[1–4]

It is often said that newborn babies, and especially preterm ones, may not respond to infection with fever. We report data indicating that newborn babies, even those born prematurely, react to infection by widening the gap between the core and the skin temperature gradients, a phenomenon analogous to that seen in feverish children and adults.

Patients and methods

A total of 81 newborn infants were studied. Fifty five were healthy babies (group A) and the remaining 26 were septicaemic (group B). The diagnosis of septicaemia was made on the clinical picture and confirmed by positive cultures from blood or cerebrospinal fluid, or both, in 21 cases and by necropsy examination in five cases. All infants were studied between the fifth and the 12th day of life and, during the whole period of the study, all premature babies and those with septicaemia were nursed in incubators in an environment the temperature of which was regulated according to their weight and extrauterine age.[5]

Temperature measurements were made by a special tele-thermometer (Yellow Spring 43TA) and always by the same person (HL). The probe of the thermometer was inserted into the rectum to a depth of 5 cm in full term, and 4 cm in preterm babies and was left there for three minutes.[6] Simultaneously the sole (skin) temperature was recorded by the special skin sensor of the instrument. The skin sensor was placed vertically over the sole and secured in place with a paper tape placed circumferentially around the foot.

During the study the 26 septicaemic infants (group B) were divided in three subgroups according to their clinical condition: group B1 comprised eight feverish infants (rectal temperature >37·8°C); group B2 comprised 11 septicaemic babies who were not feverish (rectal temperature <37·8°C) who were in comparatively good clinical condition; and group B3 comprised seven infants, who were not feverish but who were in a poor condition, and five of whom died.

Clinical data about the 81 infants are summarised in table 1. Groups A and B are

Table 1 Comparability of the 81 newborn infants

	Group A (n=55)	*Group B1 (n=8)*	*Group B2 (n=11)*	*Group B3 (n=7)*
Boys/girls	31/24	5/3	6/5	4/3
Fullterm/preterm	23/32	8/0	4/7	2/5
Mean (range) birth weight (g)	2350 (1200–3850)	3420 (2710–4880)	2310 (1250–3980)	1750 (1300–3100)
Mean (range) gestational age (weeks)	36·5 (28–40)	39·5 (38–43)	36·9 (29–40)	33·0 (29–38)
Mean age at temperature measurements (days)	8	8·9	8·5	8·9

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comparable for weight, gestational age, sex, and extrauterine age at which temperature was taken.

Results

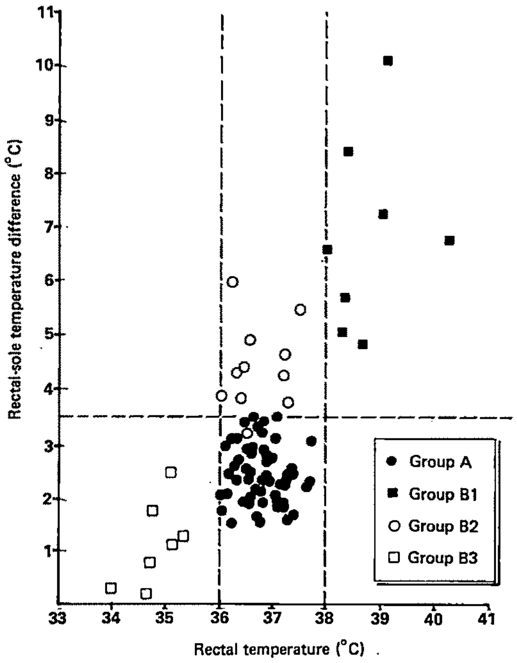
The rectal and sole temperatures, and the rectal-sole temperature differences are shown in table 2. It is of interest that all feverish septicaemic infants (group B) had a low sole temperature (31·8°C) and a big rectal-sole temperature difference (6·9 (1·5)°C), a result which was also true for group B2. In group B2, although the rectal temperature was similar to that of the healthy babies, the sole temperature was significantly lower than that of the healthy newborns; as a consequence the temperature difference (4·7 (0·8)°C) was much larger than that among the healthy babies ($p<0·001$). In contrast, the temperature difference was low in the extremely ill septicaemic babies (group B3). In this group the sole temperature was almost the same as that of the healthy infants, but the rectal temperature was low.

Plotting our data graphically (figure) we see that if the infants with low rectal temperature (group B3) are excluded, only the healthy babies had a temperature difference of less than 3·5°C. All eight feverish septicaemic babies in group B1 as well as 10 of 11 septicaemic infants with normal temperatures in group B2 had a temperature difference of more than 3·5°C. In other words, only one of the 19 (5%) septicaemic infants (groups B1+B2) had the same rectal-sole temperature difference as the healthy babies.

Interestingly, the above temperature measurements were also applied in another group of 21 infants (some born at full term and some preterm) in whom septicaemia was suspected both on clinical grounds and because of high white cell and differential counts, but in whom bacteria had not been isolated either from blood or from cerebrospinal fluid. Four of them were feverish and in good clinical condition, 14 had normal temperatures and were in relatively good clinical condition, and three had normal temperatures but were in a poor clinical condition. They all reacted in the same thermoregulatory manner as their counterparts with proved septicaemia. Four of them reacted in the same way as group B1, 11 in the same way as group B2, and three in the same way as group B3.

Table 2 Mean (SD) rectal and sole temperatures and rectal-sole temperature difference in the four groups of newborn infants (given as °C)

Group	*Rectal temperature*	*Sole temperature*	*Rectal-sole temperature difference*
A	36·7 (0·4)	34·2 (0·7)	2·5 (0·5)
B1	38·7 (0·7)	31·8 (0·4)	6·9 (1·7)
B2	36·8 (0·6)	32·1 (0·9)	4·7 (0·8)
B3	34·8 (0·4)	33·7 (0·7)	1·1 (0·8)



Rectal temperatures and rectal-sole temperature difference in all four groups of newborn infants. The horizontal dotted line shows the critical cut point of rectal-sole temperature difference of 3·5°C (see text). The vertical lines divide hypothermic, normothermic, and hyperthermic, neonates.

Discussion

Our results show that most of our septicaemic infants had a good thermoregulatory reaction to septicaemia. Those in group B1 were able to increase their body temperature and to develop fever by increasing their heat production and by reducing their heat loss. It must be stressed that all these newborns were born at full term, and that their thermoregulatory reaction was analogous to that reported by Pomerance *et al* in their feverish babies born at full term.[7]

On the other hand the 11 septicaemic infants without temperatures in group B2 reacted with peripheral vasoconstriction in their effort to minimise heat loss and to preserve their internal core temperature within the normal range. This is why their rectal temperatures were normal, in contrast to their sole temperatures which were low. This means that the babies in this group showed an adequate thermoregulatory reaction, but not as efficient as that which was shown by those in group B1. Interestingly, almost two thirds of them were premature.

The mechanism of this prompt reaction in the septicaemic newborn infants is not exactly known. In endothermic organisms, such as in newborn and adult human beings, body temperature is controlled by a rather complicated thermoregulatory system. This may be described as a 'multi-input' system, in which temperature information from several sites of the body is fed into a central 'controller'. In this controller the temperature information is compared with a reference temperature value or set point. When the integrated body temperature is above or below the set point, effective mechanisms (such as vasoconstriction or vasodilation, extra heat production or sweat secretion) are stimulated to restore the temperature to its set point.[8] During episodes of fever the set point is reset to a higher point.[2 4 9]

In this context it is not surprising that the feverish babies in group B1 reacted promptly by reducing heat loss by peripheral vasoconstriction, as their body temperature was raised. The analogous reaction of the babies with normal temperatures in group B2 deserves some comment, however. The vasoconstriction in these babies whose core (rectal) temperature did not differ from that of the healthy babies, is consistent with an upward deviation of the thermoregulatory set point. If this is true, one can speculate that some factor or factors (for example, bacterial pyrogen) may affect the set point in newborn babies. Whatever the mechanism, it is of interest that in most of the septicaemic infants, even those born prematurely, the thermoregulatory system reacted adequately.

In contrast, our seven critically ill septicaemic newborn babies with normal temperatures did not react with vasoconstriction, although their core (rectal) temperatures were low. This strongly suggests that such babies have no ability to mobilise their thermoregulatory mechanisms and are unable to conserve heat, which results in an appreciable drop of their core temperature. This must be considered as an ominous sign, as five of the seven who were unable to mobilise the mechanisms of their thermoregulatory systems, died within a few hours of their temperature measurements. An analogous thermoregulatory reaction was also shown by the 21 babies with possible (but not proved) septicaemia.

Although a high rectal-skin temperature difference may be caused by some other reasons (for example, poor cardiac output or hypovolaemia) our observations seem to indicate that this difference is a useful clinical sign in the diagnosis of septicaemia in newborn babies. The clinician should thoroughly investigate any newborn who is not feverish but has a rectal-skin temperature difference of more than 3·5°C.

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Increased concentrations of D-dimers in newborn infants

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Abstract

The concentrations of D-dimers (the D fragments of fibrinogen) were measured in blood from 15 preterm infants, and 45 born at full term, to establish normal ranges. The adult normal range is $<0{\cdot}25$ mg/l; 31 of the 60 infants (52%) had values $<0{\cdot}25$ mg/l, in 16 (27%) they were 0·25–0·5, in eight (13%) 0·5–1, in three (5%) 1–2, and in two (3%) 2–4.

D-dimer concentrations measured during the neonatal period should be interpreted with caution.

D-dimers (D fragments of fibrinogen) are produced during plasmin mediated lysis of fibrin.[1] They are more specific than fibrin/fibrinogen degradation products, which are formed during degradation of fibrinogen and fibrin.[2] D-dimers can be measured using 10 μl of plasma in five minutes from specimens taken in EDTA, heparin, or citrate by a latex agglutination technique.[3]

In adults the concentration of D-dimers is the most sensitive marker for monitoring the activity of disseminated intravascular coagulation,[3] and the adult normal range is less than 0·25 mg/l.[4] The aim of this study was to evaluate the test in newborn infants and to find out whether pregnant women had significantly increased values before delivery that might affect the concentrations in their infants.

Patients and methods

Full blood counts, coagulation screens, and D-dimers were measured in samples of venous blood taken from 15 preterm infants and 45 born at full term. The gestational ages of the preterm infants were: 27–28 weeks (n=1), 29–30 weeks (n=1), 31–32 weeks (n=10), and 33–34 weeks (n=3). The preterm infants had no serious complications of prematurity, although two received short periods of ventilation (less than 24 hours); this is our routine for infants of less than 30 weeks' gestational age. All infants received vitamin K 1 mg at birth by intramuscular injection.

For ethical reasons, only those infants born at full term who required blood sampling for other investigations (predominantly jaundice) had coagulation screens carried out. The indication for blood sampling was usually non-haemolytic jaundice (n=27); other indications included growth retardation (n=6), vomiting (n=4), passing maternal blood (n=3), 'spots' (n=3), and diabetes in the mother (n=2). Eighteen of the jaundiced infants required phototherapy; all other conditions resolved spontaneously. No infant had a positive blood culture and all were being fed enterally with either breast milk or formula milk. Blood sampling was carried out either on admission to the nursery (preterm infants) or between the first and the fifth day (infants born at full term).

MATERNAL SAMPLES

D-dimers were measured in 17 randomly selected pregnant women at full term who were in early uncomplicated labour.

LABORATORY METHODS

Full blood counts were measured using a Coulter s880 cell counter. Plasma from citrated blood specimens was assayed for D-dimers by a semiquantative latex agglutination test with a monoclonal antibody (Dimertest Latex, Agen Biomedical Ltd).[3]

Activated partial thromboplastin time was measured with Actin FS reagent (Dade, American Hospital Supply), and the normal adult range is 24–40 seconds. Prothrombin time was measured with rabbit brain thromboplastin (Manchester Thrombosis Research Foundation), and the normal adult range is 14–18 seconds. Thrombin calcium time was measured with bovine thrombin (Dade, American Hospital Supply), and the normal adult range is 9–13 seconds. Fibrinogen was measured by the Clauss thrombin clottable protein method, and the normal adult range is 1·5–4·0 g/l.

Results

The results of coagulation screening tests in the infants born at full term are shown in table 1, and the results of the D-dimers estimations are shown in table 2. All the infants had normal platelet counts ($150–400\times10^9$/l).

No association was found between the D-dimers concentration and the indication for blood sampling, the age of the infant at the time of sampling, the type of feeding, or whether the infant received phototherapy.

Table 1 Results of coagulation screening tests in 45 infants born at full term

	Mean (SD)	*Range*
Prothrombin time (secs)	16 (1·7)	13–20
Activated partial thromboplastin time (secs)	32·8 (5·1)	22–45
Thrombin calcium time (secs)	11·1 (1·2)	9–13
Fibrinogen (g/l)	2·8 (0·7)	1·6–4·2

Table 2 Concentrations of D-dimers in blood from 15 preterm infants and 45 born at full term

Concentrations of D-dimers (mg/l)	No (%) of infants		
	Born at full term (n=45)	Born preterm (n=15)	Total (n=60)
<0·25	24 (53)	7 (47)	31 (52)
0·25–0·5	14 (31)	2 (13)	16 (27)
0·5–1	6 (13)	2 (13)	8 (13)
1–2	1 (2)	2 (13)	3 (5)
2–4	0	2 (13)	2 (3)

All D-dimers concentrations in the pregnant mothers were <0·25 mg/l.

Discussion

Measurement of D-dimers concentrations has been suggested as an alternative to measuring fibrin/fibrinogen degradation products, as they have been shown to be more sensitive and specific and can be measured in as little as 10 μl of plasma. Normal adults have plasma concentrations of <0·25 mg/l. Using the more sensitive ELISA antibody test, mildly increased concentrations have also been found in normal pregnant women.[5] We were unable to confirm this finding in women in labour in whom D-dimers were measured by the latex agglutination technique.

The results of measurements of D-dimers in infants show that the range of plasma concentrations found in normal adults does not apply either to preterm infants or to those born at full term, as about half the infants had values outside the accepted adult range.

The reason for the increased concentrations of D-dimers in these infants is unknown. Transplacental passage is unlikely, because the concentrations found in 17 pregnant mothers before delivery were all less than 0·25 mg/l. Other possible explanations are that D-dimers may represent low grade activation of the coagulation system as a result of the circulatory adjustments of closure of the ductus venosus and ductus arteriosus after birth, or perhaps there is delayed renal clearance of D-dimers in the newborn. Fibrin/fibrinogen degradation products have also been found in 65% of normal term infants.[6] The results of coagulation screening tests in infants born at full term were similar to those in normal adults.

We conclude that newborn infants have ranges of concentrations for D-dimers that are different from adults, and therefore caution is necessary in their use and interpretation.

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Absent maternofetal potassium gradient at term

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Abstract

Mean (SE) umbilical venous plasma potassium concentrations at 5·5, 10, 20, and 30 minutes after the start of laparotomy at term were 4·75 (0·15), 5·95 (0·26), 6·81 (0·23), and 7·59 (0·81) mmol/kg plasma water. Maternal peripheral venous plasma concentrations were 4·30 (0·13) before and 4·26 (0·23) mmol/kg plasma water after laparotomy. Plasma calcium concentration in maternal and cord blood did not change significantly.

A reported higher umbilical than maternal venous plasma concentration of potassium at full term in the human[1 2] has been taken as prima facie evidence that the ion is actively transported across human placenta.[3] We have reinvestigated the human maternofetal venous plasma gradient reported with particular attention to time of sampling and find that, as in the rat,[4] the umbilical plasma concentration rises immediately after delivery. It appears that the reported concentration difference is likely to be artefactual.

Subjects and methods

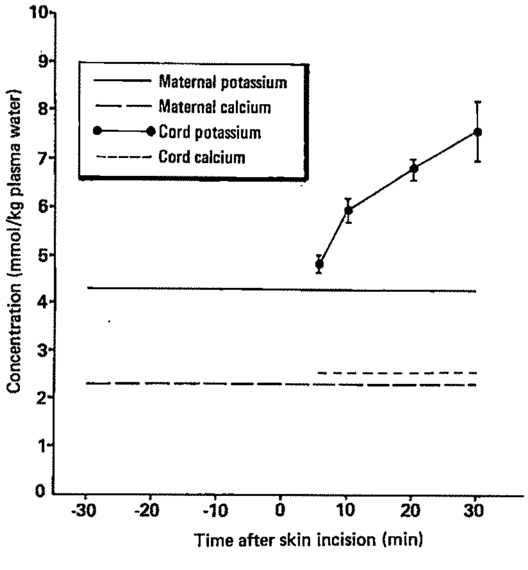
After ethical approval and maternal consent, 19 pregnant women undergoing elective caesarean section for previous section were selected on days that were convenient for study. A maternal peripheral venous blood sample of about 5 to 10 ml, from a large vein usually in the antecubital fossa using a 21 gauge needle, was taken when the patient entered the anaesthetic room about 30 minutes before surgery. A further maternal sample was taken at the end of the operation about 60 minutes after the first.

Timing of umbilical sampling was related to abdominal skin incision (time 0). At birth (mean (SE) 5 (1) minutes) the baby was lifted out and the cord was clamped in three places with clamp 1 being very near to the infant's umbilicus. Clamp 2 was applied about 5 cm away from this. Clamp 3 was applied very near to the placenta. This arrangement meant that virtually the whole length of cord was between clamps 2 and 3. The cord was cut between clamps 1 and 2 to deliver the infant. At this time (5·5 (1) minutes from incision) 3 to 5 ml of blood was withdrawn immediately from the umbilical artery and vein between clamps 2 and 3. The placenta was then removed with the clamps in situ. Further blood samples were taken from between clamps 2 and 3 and from a chorionic plate vein at timed intervals approximately 10, 20, and 30 minutes from abdominal incision. All blood samples were centrifuged immediately (within two minutes) after collecting and plasma was separated. An aliquot of the plasma was dried to constant weight at 80°C to determine plasma water content.

For potassium determinations, aliquots of plasma were diluted 200 fold in water and potassium concentration was measured by atomic absorption spectroscopy on a Perkin Elmer atomic absorption spectrophotometer. The rest of the plasma was frozen for subsequent determination of total calcium using the arsenazo-3 method in the routine biochemical laboratory. Concentrations are expressed as mmol/kg plasma water.

Results

All concentrations are expressed as mean (SE). Concentrations of potassium in cord venous plasma water at 5·5, 10, 20, and 30 minutes after incision were 4·75 (0·15), 5·95 (0·26), 6·81 (0·23), and 7·59 (0·81) mmol/kg plasma water. Maternal plasma potassium concentrations were unchanged after laparotomy (4·30 (0·13) before and 4·26 (0·23) after). Concentrations of potassium in chorionic venous plasma water at 10, 20, and 30 minutes after incision were 8·2 (0·9), 8·9 (1·4), and 11·6 (2·2), respectively



Mean (SE) potassium and calcium concentrations in plasma water mmol/kg water as a function of time of sampling. Time of abdominal skin incision taken as time 0.

The first cord sample had a mean concentration 0·37 (0·15) mmol/kg more than mean maternal value. This difference rose sharply with time (figure). Values from chorionic plate veins were substantially higher. Values uncorrected for plasma water (mmol/l) showed a similar rise.

The values for calcium confirmed the previously observed maternofetal difference (maternal 2·30 (0·05), fetal 2·54 (0·05) mmol/l/kg). There was no significant increase in cord calcium concentrations with time.

Discussion

The mean value on cord blood sample 4·5 minutes after delivery, and approximately 10 minutes after incision of the maternal abdominal wall, was 1·7 (0·2) mmol/kg plasma water higher than maternal; this is comparable with that reported in careful previous studies.[2] Five and a half minutes after abdominal incision and immediately after cord clamping, however, the cord venous concentration, although still significantly different, was only 0·37 (0·15) mmol/kg higher than maternal. It was impossible under the conditions of our study to sample cord blood at the time of abdominal incision but extrapolation by eye of the cord venous concentrations plotted in the figure back to time 0 suggest that it is highly unlikely that cord venous plasma potassium at term at the start of caesarean section is truly higher than maternal in well nourished healthy women before labour. This is probably also true in mid gestation.[5] The rise in delayed samples after cord clamping suggests leakage of intracellular red cell potassium into plasma and the greater rise seen in blood from chorionic plate vessels suggests leakage from placental parenchyma.

Fetal accretion of potassium is high and net transplacental potassium fluxes are comparable with those of sodium and chloride.[3] Its transport must be either diffusional or active. As no significant maternofetal potential difference has been reported in the human at term[2] its cord venous plasma water concentration would have to be below maternal arterial to allow for diffusional accretion. Extrapolation to time 0 in the figure suggests this is possible. Such diffusional transport would probably be via channels, perhaps specific, but in any case too small to accommodate the divalent ion calcium whose previously reported maternofetal concentration difference at term is confirmed here.[2] Alternatively active net transport of potassium must be controlled with sufficient precision to maintain maternofetal concentrations in close proximity at term. Analogy with the haemochorial placenta of the rat leads us to support the latter hypothesis,[6] but it should be noted that cord plasma concentration differences as reported here cannot be used as evidence to support such a belief.

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Anatomy and development of the fontanelle

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Abstract

Anterior fontanelles from 19 fetuses over a range of gestational ages (from 8 weeks to full term) were obtained to study the anatomy and development in relation to the application of the principles of fontanometry. The most important feature was the development of an elastic membrane by 23 weeks' gestation; a subcutaneous layer of fat had appeared by 28 weeks' gestation.

The applanation principle can be applied to a method of measuring intracranial pressure, and the anterior fontanelle is the usual site.[1] Inaccuracies in measurement may arise from variations in the elasticity of the fontanelle at different stages of gestation, or from there being too small a surface on which to fix the sensor, which is at least 1 cm in diameter. This study was undertaken to try and detect structural changes that might explain inaccuracies in fontanometry. Many methods have been used to measure fontanelle size and we used a modification of Scammon's technique.[2]

Material and methods

Nineteen fontanelles were examined over a developmental period ranging from a fetus of 8 weeks' gestation to a full term neonate who died four weeks after birth.

The surface area of the non-macerated fontanelle was measured by placing soft paper over the previously dried skin, palpating the corners of the fontanelles and rubbing over these corners and edges with a soft pencil. This produced a crude 'brass rubbing' of the outline of the fontanelle. This was transferred to graph paper by pricking through it with a pin. The squares within the outline were counted, and the area in square centimetres recorded.[2] In addition, the shortest diameter of each fontanelle (Elasser's diameter) was measured, as during fontanometry it is important that the sensor does not touch the edges of the fontanelle.

Blocks for microscopy were taken by excising the fontanelle together with a 0·5 cm rim of bone. A slice was trimmed across the middle of the block to include bone at either side. In the case of a necropsy fetus a coronal skin incision was made, and this incision formed the anterior edge of the block that was taken coronally across the posterior half of the fontanelle. The skin was sutured to the rest of the block before processing. Blocks were decalcified with 10% formic acid, routinely processed and embedded in paraffin; 3 to 5 micron sections were stained with haematoxylin and eosin, and elastic van Gieson's stain.

Results

The gestational ages of the fontanelles are shown in table 1.

ANATOMY

The fontanelle was defined laterally by the bony skull plates and so could not be delineated until 12 weeks' gestation when these first appeared.

From the lateral to the medial edges, the bony plates gave way to islands of bone and then to aggregates of plump fibroblasts surrounding pink osteoid like material. At the centre of the fontanelle no precursors of bone were present. The vertical structure of the fontanelle was best considered in levels: level 1 comprised the skin; level 2 comprised the connective tissue below level 1 and the bony skull plates; level 3 was the connective tissue between level 2 and the dura; and level 4 was the dura (fig 1a).

At 8 weeks' gestation level 1 (the skin) was a single layer of flat epithelial cells. This became stratified epithelium by 16 weeks, with a dermis containing hair follicles. By 19 weeks occasional sebaceous glands could be seen. By 23 weeks small clusters of fat cells had developed in the dermis. By 28 weeks a continuous layer of subcutaneous fat had developed; no major structural alteration occurred after this time. In levels 2 and 3 there were slight increases in the density of the collagen as gestation increased. Level 4 (dura) consisted of a single layer of cells until 28 weeks' gestation, when two layers of cells became visible. The distribution of elastin changed with increasing gestation; it was first seen as diffuse strands within dural vessels at 16 weeks, but a well defined elastic lamina had formed by 19 weeks' gestation. At this time wisps of elastin were seen in the connective tissue between levels 1 and 2. By 23 weeks a well defined elastic membrane had developed at this point (fig 1b). This membrane was present

Table 1 Gestational ages of the 19 fontanelles studied

Gestational age (weeks)	*No of fontanelles*
8	1
12–16	5
19–22	4
23–27	4
28–52	5

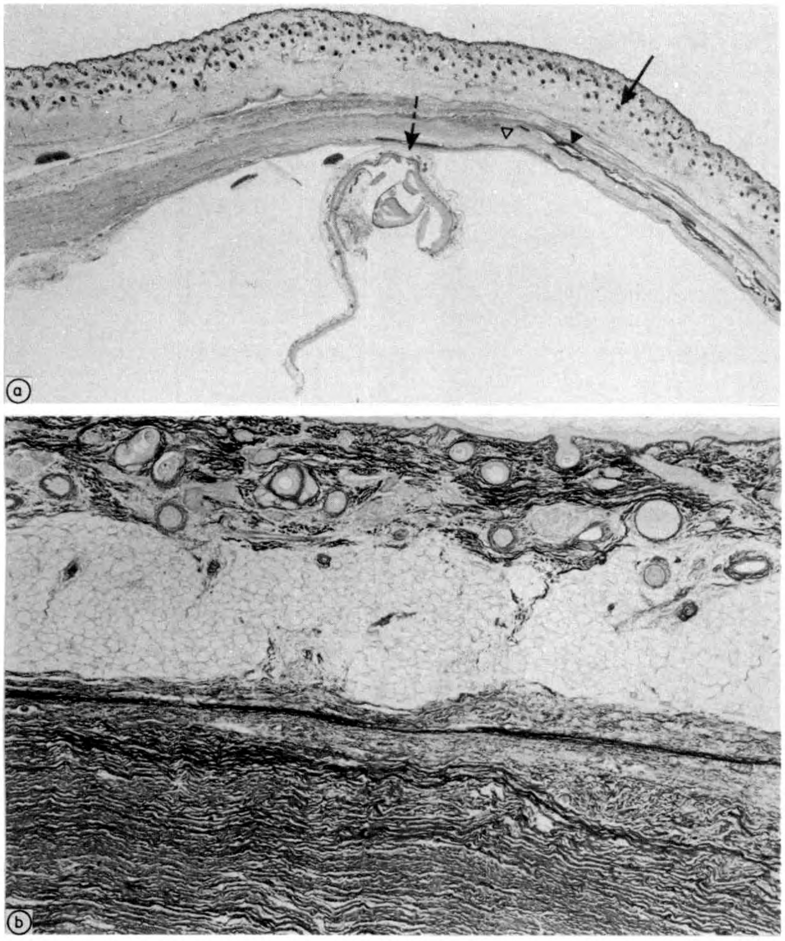

Figure 1a Section of the fontanelle showing four levels: (i) skin (solid arrow); (ii) connective tissue containing skull plates (solid triangle) and islands of bone; (iii) connective tissue beneath second layer (open triangle); and (iv) dura (dotted arrow), with adherent superficial cerebral cortex (Haematoxylin and eosin, magnification×8·5). Figure 1b Section of 28 week old fontanelle showing elastin membrane and fat layer (elastic van Gieson's stain, magnification×65).

in all fontanelles of 23 weeks' gestation and more but the intensity varied. By 26 weeks' gestation septa of elastin had appeared in the subcutaneous fat.

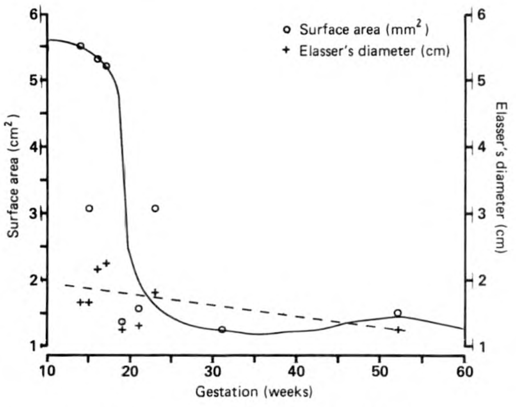

Figure 2 Graph of variation of surface area and minimum diameter of fontanelle with gestation.

Table 2 Changes in mean surface area and Elasser's diameter with gestational age

Gestational age (weeks)	*Surface area (mm^2)*	*Elasser's diameter (cm)*
14	5·50	1·65
15	3·06	1·65
16	5·31	2·15
17	5·19	2·25
19	1·37	1·25
21	1·56	1·30
23	3·06	1·80
31	1·25	Not measured
52	1·50	1·25

SURFACE AREAS

As gestation increased surface area decreased exponentially. Elasser's diameter hardly changed at all with respect to the size of the 1 cm sensor (fig 2, table 2).

Discussion

Important changes in the structure of the fontanelle were noted—in particular, the formation of a well defined elastic membrane by 23 weeks, although the density of this varied among specimens. Fat became apparent as early as 23 weeks, and had formed a thick subcutaneous layer by 28 weeks. If fontanometry is carried out on babies born at full term, these structures would be fully developed and under normal conditions little variation would be expected. More variation in density of the elastic membrane was seen in fontanelles between 23 and 28 weeks, and so we postulate that changes in the density of these structures may cause inaccuracies in fontanometry in younger fetuses. The second factor in assessing accuracy of measurements is size of the fontanelle, and it can be seen from the results that in the age group in which fontanometry was applied the size was relatively constant at different gestational ages. The fontanometry sensor should fit on to the fontanelle of a baby as young as 3 weeks of age.

The eventual closure of the fontanelle presumably occurs by transformation of fibroblasts into osteoblasts. These form bony islands that join to the main skull plate. A study of a larger number of fontanelles at each gestational age would provide more data about individual variations in anatomy, but this study has shown that an elastin membrane does develop between the skin and the plane of the bony plates. We believe that this might be important in assessing the results of fontanometry in younger preterm fetuses.

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Mosaic trisomy 16 in a live newborn infant

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Abstract

Trisomy 16 is thought to be incompatible with fetal survival. A boy with mosaic trisomy 16 who lived for 11 weeks is reported. Chromosome analysis was carried out on skin fibroblasts grown during life and confirmed on samples taken at necropsy. We believe that this is the first report of mosaic trisomy 16 that has been confirmed by cytogenetic banding.

Trisomy 16 is the most common autosomal anomaly seen in early aborted fetuses, but has been thought to be incompatible with full embryonic development; empty sacs and disorganised embryos are usually seen.[1] There are reports of putative full and mosaic trisomy 16 but none confirmed by cytogenetic banding studies. We report an abnormal baby boy born alive with mosaic trisomy 16 who survived for 11 weeks.

Case report

A baby boy was born at 39 weeks' gestation to healthy Chinese parents. During the pregnancy symmetrical intrauterine growth retardation and polyhydramnios were noted; delivery was by caesarean section because of fetal distress and the growth retardation.

The baby was initially cyanosed with regular shallow respiratory efforts and a heart rate of 60 beats/minute; he responded well to oxygen given by face mask. Craniofacial and musculoskeletal dysmorphic features were immediately apparent as was clinical growth retardation; he weighed 1590 g at birth, was 40 cm long, and had a head circumference of 31·5 cm (all below the third centile). The abnormalities noted are listed in the table; the infant is shown in the figure.

The baby required ventilatory support for the first three weeks because of respiratory distress, and was then maintained with oxygen given by headbox for a further three weeks. The respiratory insufficiency was thought to be caused by restriction from kyphoscoliosis. A ventilation-perfusion lung scan carried out at 8 weeks showed equal ventilation bilaterally, but with reduced ventilation in the upper zones. There was a discrepancy between ventilation and perfusion in the right upper zone, which was underperfused.

A loud pulmonary second sound and forceful left parasternal impulse were noted at birth; a pansystolic murmur was audible at the lower left sternal edge from 4 weeks. An echocardiogram showed a small perimembranous ventricular septal defect, which seemed to be closing by apposition of the tricuspid valve tissue.

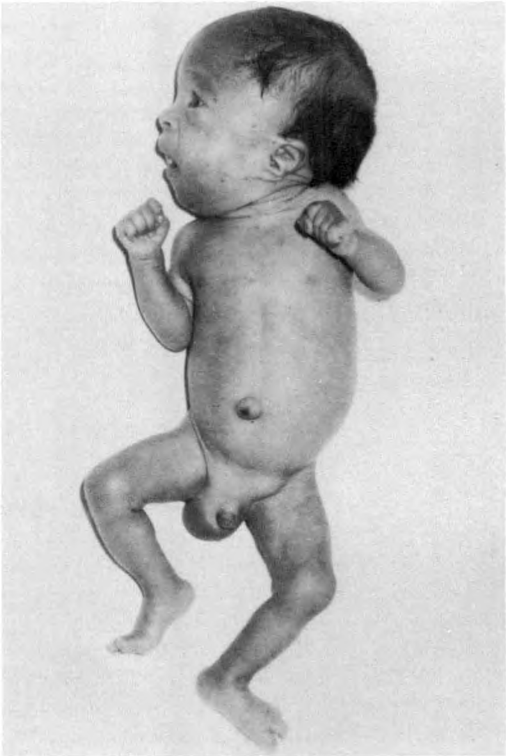
Phenotypic abnormalities

Craniofacial:
- Cranial asymmetry
- Prominence of left side of forehead
- Abnormal scalp hair patterning with high frontal hairline on left
- Hypertelorism
- Swelling of right upper eyelid giving appearance of ptosis and micro-ophthalmos
- Partial coloboma left iris
- Short nose, flat nasal bridge
- Thickened upper lip with groove in upper gingival margin, left of midline
- Preauricular pit
- Low set malformed auricles

Musculoskeletal:
- Thoracolumbar scoliosis convex to left
- Prominent left hemithorax
- Hypoplastic left nipple
- Single palmar crease, left hand
- Middle finger overlapping index
- Wide space between first and second toes
- Dorsiflexed toes
- Bilateral talipes calcaneovalgus

Genital:
- Undescended testis
- Left inguinal hernia
- Non-communicating hydrocele

Cardiac:
- Ventriculoseptal defect



Appearance of infant with mosaic trisomy 16

The infant was fed initially by nasogastric tube, and subsequently by bottle from the age of 8 weeks. Despite a maximal milk intake his weight gain was slow and erratic. He gained just

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over a kilogram during the 11 weeks of his life, and weighed 2610 g at the time of his death.

He was always socially unresponsive and was never seen to smile. He was hypotonic with no head control ('rag doll-like'). He was visually attentive and had normal non-corneal electroretinograms, with bilateral cortical visual evoked potentials to flash stimuli. He did not respond to sound; auditory evoked potentials at eight weeks were inconsistent. An electroencephalogram showed no abnormality.

At 11 weeks his inguinal hernia became difficult to reduce and surgical repair was indicated. He was by that time clinically stable, and had been breathing air spontaneously for five weeks. The endotracheal intubation for general anaesthesia was extremely difficult, and the patient became hypoxic and had a cardiorespiratory arrest. Resuscitation was unsuccessful.

Necropsy was performed at the request of the coroner and left sided hypertrophy of the cornua of the hyoid bone and thyroid cartilage was found, together with pronounced hypertrophy of the left arytenoid cartilage which seemed to have caused partial obstruction of the glottis. There was a deep cleft between the hypertrophic left arytenoid area and the epiglottis. The vocal cords were asymmetrical, particularly on the left, and there was stenosis in the midsection of the trachea. A small aberrant left bronchus that was barely patent was present above the left main bronchus, and the right bronchus was elongated. There were no other findings of note except for the small perimembranous ventriculoseptal defect previously noted, and the dysmorphic features.

Cytogenetic studies

Chromosome analyses using G banding were carried out on 72 hour lymphocyte cultures. A total of 20 metaphases were analysed and no abnormality was found, suggesting a karyotype to be 46, XY.

The chromosomal analysis was repeated using fibroblast cultures from a skin biopsy specimen taken from the left thigh during the second week of life. G banding of the chromosome preparations indicated two different cell lines. Of a total 21 metaphases examined, six showed a normal male karyotype, 46, XY. The remaining 15 metaphases were trisomic for the whole of chromosome number 16, with a chromosome count of 47. The proband karyotype was 46, XY/47, XY+16.

The cell line which was trisomic for chromosome 16 was less stable in culture than the normal cell line. The proportion of trisomic cells to normal cells decreased with increasing time in culture, especially after subculturing the fibroblasts. The trisomy 16 mosaicism was confirmed in fibroblasts taken from skin and muscle biopsy specimens taken at necropsy. Roughly half the fibroblasts were trisomic for chromosome 16, and the rest showed a normal karyotype. Further tissues were not cultured for chromosomal studies and so the differential distribution of trisomic cells could not be examined. Studies of both parents showed normal karyotypes.

Discussion

We believe that this is the first report of a mosaic trisomy 16 confirmed by cytogenetic banding, and we have described phenotype abnormalities in addition to those noted previously in potentially similar cases.

Trisomy 16 has been reported as the most common autosomal trisomy in aborted fetuses. There have been isolated reports of 16− mosaicism in adults; a physically and mentally normal Japanese father of an aborted fetus that was trisomic for chromosome 16 had 3% of his white cells and 15% of his skin fibroblasts trisomic for chromosome 16.[2] Some of the abnormalities that were seen in our proband—including kyphoscoliosis, breast hypoplasia, and asymmetric musculoskeletal development—have been described previously,[3] but in these reports of mosaic trisomy 16 and in two of full trisomy 16 chromosome analysis was carried out without modern banding techniques.[4 5]

There have been reports of infants surviving with trisomy of either 16p or 16q material confirmed by GTG banding[6]; partial trisomy 16 may also occur with familial 16:21 translocation. Patients with partial trisomy 16 all have multiple congenital anomalies and with one exception have died within the first year of life. As both trisomy 16p and 16q are compatible with intrauterine life and a limited degree of postnatal survival Roberts and Duckett concluded that it must be the combined effects of additional long and short arms which render the full trisomy invariably lethal.[6] It is noteworthy that trisomy 16 has been detected in chorion villus biopsy specimens from a number of cases in which the fetus itself was unaffected, indicating that the mosaic is formed in the early embryo and that the trophoblast can function normally in the presence of this trisomy. Our proband has some features in common with those seen in partial trisomies, namely skull asymmetry, auricular abnormalities, single palmar crease, cryptorchidism, inguinal hernia, ventriculoseptal defect, failure to thrive, and developmental retardation. Other features of this infant that have not been noted previously include coloboma, abnormal patterning of scalp hair, and asymmetry of laryngeal structures.

This infant had a higher proportion of abnormal cells than suggested by the unconfirmed reports of mosaic trisomy 16. It remains to be determined what proportion of abnormal cells can exist before a mosaic trisomy becomes lethal, or if a full trimsomy 16 is compatible with survival. Consideration of this baby's anomalies may assist in delineation of a phenotype for the trisomy of chromosome 16 material.

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CURRENT TOPIC

Diagnosis and management of fetal growth retardation

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What is fetal growth retardation?
Fetal growth retardation is best defined as the failure of a fetus to achieve its growth potential.[1] Though this is the theoretically correct definition, in practice various less precise criteria have been used, including: measures of absolute size such as low birth weight (less than 2500 g), and very low birth weight (less than 1500 g); and measures of relative size (small for gestational age), variably defined as less than the third centile, less than the tenth centile, and less than two SD below the population mean for gestational age and sex.

These practical criteria have limitations, and comparison of studies using different definitions of fetal growth retardation is difficult. Because normal fetal growth is under intrinsic and extrinsic control by, for example, genetic, endocrinological, and nutritional factors, there is considerable variation in the incidence of pathological fetal growth depending on the population studied.[2] In addition, not all 'small' babies by these definitions are pathologically grown, and conversely, some babies that are 'not small' may not have achieved normal pathological growth. Finally, the confounding effects of preterm birth are not easy to disentangle.

There is conflicting evidence as to whether the fetus that is small for gestational age has a higher mortality than the fetus that is appropriately grown when one allows for confounding variables such as congenital abnormality, intrauterine viral infection, and gestational age.[3 4] The association between fetal growth retardation and mortality is not yet established and the question will only be resolved if sufficiently large numbers are analysed to take account of these variables.

Clearly there will be increased morbidity in fetuses that are small for gestational age with either a congenital anomaly or an intrauterine viral infection, the exact prognosis depending on the diagnosis. Even when these problems are excluded, however, in contrast to the mortality data, the fetuses that are small for gestational age do seem to be at increased risk of both short and long term morbidity.[3 4] When one allows for confounding variables such as gestational age, race, gender, and socioeconomic group, there may be as much as six times the risk of neurodevelopmental handicap at one year of age in these infants compared with these infants who have grown appropriately.[3] In addition, the effects may vary with the severity,[4] the duration,[5] and the type of fetal growth retardation, namely the degree of retardation of fetal head growth.[6]

Which fetus is at risk of developing growth retardation?
A number of epidemiological factors are associated with an increased risk of a fetus being small for gestational age. These include fetal sex, multiple pregnancy, birth order, socioeconomic group, maternal height and weight, ethnic group, and interpregnancy interval.[7] Several authors have used these associations to try and predict the risk of the subsequent occurrence of a fetus that is small for gestational age. For example, Galbraith *et al* in a study of 8030 babies,[8] claimed a positive predictive value of their risk scoring method of 9·8% and a false negative rate of 2·3% (sensitivity 69·1%, specificity 67·1%). Such risk scores have not been widely implemented; they are cumbersome to use, they have a poor predictive value, and they do not always predict the fetus that is pathologically small for gestational age.

A more practical approach is to identify pregnancies with specific risk factors for a fetus being small for gestational age compared with the normal population, and then to measure the growth of those fetuses serially with ultrasound to identify those that actually develop pathological growth. The following are examples of such risk factors.

Severe maternal malnutrition resulting in reduced energy intake and pathological fetal growth is seen during major famines, but is only likely to be encountered in developed countries if the mother develops a rare condition such as pancreatitis, active inflammatory bowel disease, or an eating disorder, or has a bowel resection. In such cases restricting the mother's dietary intake to less than 3·77 MJ/day for six months will result, on average, in a fetus that is 240 g lighter than the fetus whose mother ate normally.[9]

Alcohol consumption in pregnancy is a cause of fetal growth retardation. For example, the odds ratio for a fetus being small for gestational age if a mother takes two drinks a day is 1·62 (95% confidence interval (CI) 1·26 to 2·09). This risk rises progressively with the number of drinks consumed.[7] Mothers addicted to opiates have five times the risk of fetal growth retardation compared with controls.[10]

Essential hypertension alone is not associated with a greater likelihood of a fetus being small for gestational age, though this is contested by some authors.[9] The risk of a fetus being small for gestational age is doubled if the mother has proteinuric pre-eclampsia alone, and quadrupled if this is superimposed on pre-existing hypertension. A mother with cyanotic cardiac disease has a risk of a fetus being small for gestational age that is ten times that in the normal population. In chronic maternal renal disease the risk is related to the degree of renal dysfunction and hypertension.[10] Sickle cell disease (and its variants), and systemic lupus erythematosus, are two other important diseases that are associated with a greater likelihood of fetal growth retardation.[9 10]

A raised maternal serum α fetoprotein concentration in the first half of pregnancy, not associated with fetal abnormality, carries an increased risk of a fetus becoming small for gestational age later in the pregnancy.[11] Other biochemical tests, largely discarded over the past 15 years, perhaps warrant further examination as predictors of fetal growth failure before it becomes clinically or ultrasonically apparent. Assays of human placental lactogen, oestriol, and Schwangerschaftsprotein-1 all have a sensitivity and positive predictive value for a fetus being small for gestational age of between 30–50%.[12]

The ability of Doppler ultrasound to predict the fetus at risk of growth retardation has been studied using recordings from both the uteroplacental and fetal circulations. In an early pilot study of uteroplacental waveforms in 126 pregnancies at 16–20 weeks, an abnormal waveform predicted 68% of pregnancies with fetal growth retardation with a specificity of 65%, positive predictive value of 26%, and a negative predictive value of 92%.[13] These encouraging results need to be confirmed by larger studies. Umbilical artery Doppler recordings later in pregnancy are relatively poor predictors of a fetus that is small for gestational age, though they do seem to be better at predicting subsequent fetal asphyxia.[14]

Finally, maternal smoking is one of the most common contributing factors to fetal growth retardation (up to 40%) in developed countries.[9] The babies born to mothers who smoke cigarettes in pregnancy are approximately 250 g lighter than those born to mothers who do not smoke.[7 15] Ironically the habit is too widespread to make it a practical proposition to carry out serial ultrasonic growth scans on all fetuses of mothers who smoke and furthermore, by itself it is a poor predictor of a fetus being small for gestational age.

Can we prevent fetal growth retardation?

Giving up smoking during the first half of pregnancy, and stopping or reducing the drinking of alcohol during pregnancy both improve fetal growth.[10] In areas of famine where severe maternal malnutrition results in fetal growth retardation, the effect can be prevented by giving the mother more to eat.[9] It has been suggested, though not universally agreed, that conversion of opiate addicts to low dose methadone for the duration of pregnancy results in delivery of infants with weights comparable with those of non-addicted women of similar socioeconomic class.[15]

A patient who has hypertension before the onset of pregnancy should have the hypertension controlled. The evidence suggests that control of blood pressure before pregnancy reduces the risk of having a fetus that is small for gestational age in the subsequent pregnancy, whereas treatment of hypertension during pregnancy has no appreciable effect on fetal growth.[16] The high risk of fetal growth retardation if the mother has cyanotic heart disease is roughly halved if the patient has corrective surgery before pregnancy.[9] For the patient with chronic renal disease, control of hypertension before pregnancy would seem to be of benefit, the most remarkable reduction of risk, however, is achieved by renal transplantation provided that proteinuria and hypertension do not continue after transplantation and that there is no evidence of graft rejection and renal function is normal.[17] The result is also dependent on the patient taking small doses of immunosuppressive drugs such as prednisolone and azathioprine. Cyclosporin, on the other hand, seems to be associated with an increased risk of a fetus that is small for gestational age. In patients with sickle cell disease there is no information about whether prevention of recurrent crises by adequate hydration and prompt treatment of infection reduces the likelihood of a fetus that is small for gestational age.[15]

Though there is good theoretical and experimental evidence that bed rest should improve the uteroplacental blood supply and thus reduce the risk of fetal growth retardation, there is no convincing evidence of its value in practice.[15]

Treatment with low doses of aspirin, in contrast, holds out more hope for prophylaxis of fetal growth retardation. There have now been several reports that such treatment has resulted in a lowering of the incidence of pre-eclampsia, a fetus that is small for gestational age, or both, in at risk pregnancies.[18 19] The results of the Medical Research Council (MRC) multicentre trial of treatment with low doses of aspirin are awaited with interest.

Can we identify the fetus with growth retardation?

Clinical inspection and palpation have sensitivity of 44%, a specificity of 88%, and a positive predictive value of 29% in predicting the fetus that is small for gestational age.[20] Measurement of fundal-symphysial length is only slightly better with a sensitivity between 62–86%, a specificity between 79–89%, and a positive predictive value between 18–79%.[20 21] Though clinical measures tend to be unreliable screening tests, however, the more accurate results have come from studies of high risk pregnancies.[21]

Fetal ultrasonographic measurement remains the best method by which fetal growth retardation is identified. The most commonly measured fetal dimensions are biparietal diameter, head circumference, abdominal circumference, and length of femur. They are used either separately or together. All studies show that the abdominal circumference is the single most effective measurement for monitoring fetal growth and predicting fetal size, as it is reduced in both symmetrical and asymmetrical types of fetal growth retardation. Head and femur measurements are often unaffected in asymmetrical fetal growth retardation.[22] Though serial fetal measurements and the plotting of growth trajectories (using charts appropriate for a given population) are the best methods of diagnosing pathological fetal growth, single measurements are less helpful.[21] Prediction of fetal weight from a single measurement of abdominal circumference is associated with an error of about 16%.[22] This error may be greater at the extremes of size with a tendency to overestimate the size of small babies and to underestimate the size of big babies.[21] Formulas for predicting fetal weight that incorporate abdominal circumference plus one or more other measurements reduce the error of the predictions.[22]

Serial ultrasound recording of fetal growth is possible in a small number of high risk pregnancies, but it is not feasible as a screening procedure for women who are not obviously at risk. A number of studies have been undertaken to assess the effectiveness of ultrasound measurements on one or at most two occasions in pregnancy to screen for the fetus that is small for gestational age in normal and low risk populations. The results are difficult to compare because of variables such as gestational age at the time of the scan, the prevalence of fetuses that are small for gestational age in the study population, the definition of small for gestational age, the measurements made, the skill of the observers, and the equipment used. The sensitivity varies from 43–94%, the specificity from 84–92%, and the positive predictive value from 20–70%.[20] These figures are not dissimilar from the ranges quoted for measurements of fundal-symphysial length. Indeed, in a comparative study in the same group of women, there was no difference between a single measurement of abdominal circumference in the last trimester and serial fundal measurements of length, though combining the two improved the accuracy.[22]

Of perhaps greater relevance is whether routine clinical or ultrasound screening for fetuses that are small for gestational age in the normal population is of any value in terms of outcome. For example, there have been five randomised controlled trials of ultrasonographic screening; only one of the five reported an improved outcome, the other four failing to show any benefit.[20]

What should we do when fetal growth retardation is present?

The first requirement is careful counselling. It is clear that there are limitations in our ability to identify the fetus that is small for gestational age accurately, and it is often impossible to distinguish the fetus that is pathologically growing from the fetus that is constitutionally or genetically small. A balance must be maintained in what we say to avoid misleading and confusing the mother therefore, so that we do not cause unnecessary anxiety, but succeed in conveying concern where appropriate.

When pathological growth is suspected, further evaluation is necessary both to confirm the diagnosis and to establish the aetiology. The first requirement is to confirm that the fetus is normal. Though every routine scan should include such an assessment, it is especially important if there is the possibility of fetal growth retardation.[23] Further ultrasonic documentation of growth may be helpful in distinguishing the genetically or constitutionally small fetus (whose growth should follow the normal centile trajectories) from the pathologically growing fetus (whose growth, especially of the abdomen will fall away from expected trajectories).[23] Because of the errors associated with ultrasonographic measurements, it is probably wise to ensure that the interval between growth scans is not less than two weeks. Invasive procedures have been used to evaluate the causes of growth retardation. Amniocentesis is of limited value, but placental biopsy (for rapid karyotyping) and umbilical cordocentesis (for karyotyping and viral serology) are being increasingly done.[15 22] These procedures are not without risk, however, and there are no clear guidelines as to when they are appropriate.

Specific management depends upon the cause. In the case of fetal abnormality this will be determined by the diagnosis and prognosis, the gestational age, and the parents' wishes. In the case of viral infection the prognosis is not always easy to predict, which makes counselling more difficult because it is difficult to detect which organs are affected in utero, and though the baby may be normal (though small) at birth, subtle damage (for example, hearing loss or moderate mental retardation) may not be identified until later in infancy.[24]

In other cases, such as uteroplacental vascular disease, recurrent bleeding, or idiopathic growth retardation, in which the baby is normal and there is no viral disease, the current approach to management can be considered under four headings.

GENERAL MEASURES

Giving up smoking and alcohol in the first half of pregnancy have both been shown to result in improved fetal growth, compared with those mothers who continue with these habits.[10] As discussed above, despite good theoretical reasons the value of bed rest in reducing the risk of fetal growth retardation remains to be proved.[15]

ASSESSMENT OF FETAL WELLBEING

Apart from confirming the diagnosis of fetal growth retardation, serial ultrasound measurements of fetal growth give an indication of its severity and may indicate when fetal growth ceases.[23] Over the last decade evaluation of fetal health had largely been undertaken by biophysical methods, which have thus replaced biochemical methods.[12] Most of the biophysical methods were developed after retrospective evaluation of their ability to predict fetal asphyxia or death. They were introduced with great enthusiasm, yet most have not been subjected to prospectively controlled clinical trials. They are generally better at confirming normality (high specificity and negative predictive value) than in identifying abnormality (low sensitivity and positive predictive value).[21]

The importance of maternal perception of fetal movements has been recognised for a long time, and has been used in various methods of assessing fetal wellbeing. The value of 'kick charts' has recently been questioned by a study of nearly 70 000 pregnancies in which the use of the charts was not associated with any lowering of the rate of fetal loss.[25] Whether they would produce better results in a high risk group has not been assessed.

Measurement of the fetal heart rate at rest is currently the most widely used method of fetal assessment. Its value in predicting fetal hypoxia was well reviewed by Devoe *et al.*[26] Using various outcome measurements of fetal hypoxia, the measurement of heart rate at rest had a sensitivity of 50%, a specificity of 90%, a positive predictive value of 40%, and a negative predictive value of 95%.

The fetal biophysical profile score has two underlying principles: firstly, that the fetus subjected to chronic asphyxia will in time undergo changes as a result of the effects of the hypoxia on renal function (amniotic fluid volume) and cerebral function (fetal tone, movements, breathing, and variability of heart rate); secondly, by combining the five measurements in one assessment a more accurate prediction of fetal risk can be obtained than from any one measurement alone. The overall gross perinatal mortality rate in high risk monitored patients is about 7:1000 (corrected for lethal abnormalities and rhesus disease it is below 2:1000) compared with an untested population figure of 14:1000.[27] The biophysical profile score gives higher incidence of normal results (97·5%) than measurement of the fetal heart rate at rest (80%) and a lower false negative rate (the risk of a fetal death occurring within one week of a normal result from the biophysical profile score is 0·68/1000 tests compared with 4/1000 for measurement of fetal heart rate).[27]

Doppler recordings from fetal vessels (umbilical artery, descending aorta, and cranial arteries) seem to be sensitive indicators of the fetal circulatory state. The absence of end diastolic recordings in the umbilical artery, or descending aorta, or both, in growth retarded fetuses seem to be associated with a high incidence of fetal hypoxaemia and the subsequent development of perinatal asphyxia.[14] The clinical management appropriate for growth retarded fetuses with abnormal blood flow, however, has to be established in prospective randomised studies before any recommendations for general clinical use can be made.

Cordocentesis can be used to measure fetal blood gas tensions and it is attractive to consider this to generate a rational approach to the management of growth retarded fetuses. Though this approach has been strongly advocated by some authors,[15 22] there are no prospective data linking fetal cord blood gas values to outcome, either in the short or the long term. What are the indications for cordocentesis? If the tensions are normal, when should the procedure be repeated? Furthermore, cordocentesis is itself a potentially hazardous procedure. Until the outcome data from prospectively conducted intervention trials have been produced, cordocentesis for fetal blood gas analysis should not be introduced into clinical practice.

SPECIFIC TREATMENTS

Aspirin has already been mentioned as possibly preventing fetal growth retardation. There is also some evidence that it may limit or improve fetal growth retardation once present. Trudinger *et al* showed in a double blind placebo controlled study that women with high umbilical arterial velocities given 150 mg aspirin daily delivered babies over 500 g heavier than the control group.[19] In that study, however, though most of the women were recruited because their fetuses were small for gestational age, there were no details of fetal size at entry.

β-Sympathomimetics increase fetal birth weight in animals primarily by improving uterine blood flow. There is, however, no clear evidence of their value in human growth retardation.

Solcoseryl is a haemodialysate containing no protein or antigens derived from calf blood. Theoretically it may improve fetal growth by effects both on fetal metabolism and on the maternal circulation. A small study of its use in pregnancies complicated by fetal growth retardation reported a tenfold lower incidence of babies that were small for gestational age in the treated group.[28] Allylestrenol is a synthetic progestogen that has been used in the management of both threatened abortion and of fetal growth retardation. It may improve the maternal uteroplacental circulation either by α receptor blockade or by antiplatelet action. More study is required of its value in fetal growth failure.[15]

The treatment of fetal growth retardation by nutrient supplementation (carbohydrate, lipids and amino acids, given both singly and in combination either to the mother or to the fetus) has been studied mainly in animals with experimentally induced growth failure, and with varying success. There are few data about humans.[15]

Maternal hyperoxygenation is another possible treatment for severe fetal growth retardation that requires further evaluation. In a preliminary study of 19 fetuses with severe growth retardation and abnormal umbilical artery Doppler measurements and fetal hypoxaemia, humidified oxygen (55%) was given to the mothers for 24 hours a day. In four cases there was a rise in the calculated fetal thoracic aorta velocity for several days or weeks, and all four infants survived. In six cases there was no improvement in aortic velocity; among the six cases, there was one fetal death and four neonatal deaths. In the remaining nine cases initial improvement in aortic velocity was followed by a fall; there was one fetal death, four neonatal deaths, and four infants survived the neonatal period.[15 29] Though these preliminary data suggest that maternal hyperoxygenation may improve fetal oxygenation and delay delivery until the fetus may survive outside the uterus, the benefit has not yet been proved, and the risks to the mother will have to be carefully monitored.

DELIVERY

The timing and method of delivery of the fetus with growth retardation are influenced by the factors discussed above. It may be relatively straightforward, for example, when the fetus has a lethal abnormality. When the fetus is normal, however, the decisions are not as easy, mainly because the necessary prospective randomised controlled studies with long term follow up into childhood have not been undertaken. A number of unanswered questions remain, therefore. For example, the current practice is to deliver the fetus with growth retardation once the biophysical criteria become abnormal or 37 weeks' gestation is reached, whichever is sooner. But is the fetus with growth retardation but normal biophysical measurements better delivered before the measurements become abnormal, even though that may expose the baby to the complications of preterm delivery? What is the role of cordocentesis in resolving this? What are the criteria for allowing vaginal delivery to be attempted?

Undoubtedly when vaginal delivery is undertaken the fetus with growth retardation is at greater risk of intrapartum hypoxia. The current practice is to monitor the fetal heart rate continuously during labour, and if this becomes abnormal to assess fetal capillary pH and expedite delivery if it is less than 7·2. In addition, many clinicians consider the presence of meconium in the liquor as an ominous sign. In general, normal heart rate patterns and the absence of meconium are indicative of fetal wellbeing during labour. When intrapartum acidosis occurs in the pathologically grown fetus it is usually at a faster rate than in the normally growing baby. In such cases, when the fetal heart becomes abnormal it is usually characterised by variable or late decelerations.[30] Fetal heart rate monitoring and pH testing are, however, far from perfect ways of assessing intrapartum fetal health. They are associated with a relatively low positive predictive value and sensitivity in predicting fetal asphyxia, in whatever way it is defined.[30,31] There is a growing need for improved methods of continuously assessing the health of the fetus with growth retardation during labour.

Conclusions

The diagnosis and management of the fetus with growth retardation remain high priorities in obstetric care. Current practice is, however, far from ideal. There is a need for a universally agreed definition of fetal growth retardation. There are limitations in our ability to diagnose pathological fetal growth. Many methods of predicting, assessing, and treating the fetus with growth retardation have been proposed. The outcome, however, both in the short and long term, of properly constructed prospective studies should be awaited before such strategies are introduced into clinical practice.

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PERINATAL LESSONS FROM THE PAST

Charles White (1728–1813) and natural birth

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Charles White was born in Manchester in 1728. After an apprenticeship with his father Thomas, an eminent surgeon and man-midwife, he trained with William Hunter in London, and after a further year's study in Edinburgh returned to Manchester where he helped to found the Manchester Infirmary in 1752. Such was his distinction that he was admitted to membership of the Royal Society at the age of 33. In 1773 he published his most important work, a treatise on the management of pregnant and lying in women.[1] This book written 70 years before Pasteur and Semmelweis is especially remarkable for its observations on puerperal fever. White recognised that the condition was contagious and that there was a need to isolate affected patients and to disinfect their rooms and bedding after use. Above all he stressed the importance of prevention by strict cleanliness, good ventilation, and the encouragement of the free drainage of lochia by nursing the mother in a sitting position and by early ambulation. After 21 years' practice and at a time when one in 25 parturient women died from puerperal sepsis, he was able to claim with modesty that he had never lost a patient from this disease.

The second half of the 18th century may be regarded as the golden age of British obstetrics. Men like White preferred whenever possible to observe and admire nature rather than intervene, except when necessary. As he wrote:

'I might say it is inconceivable that Nature should suffer her most important process to be the least complete, and that she should need the help of art in an operation, almost prior to art itself. In her inferior productions we find that, in fact, she does not require it. The process of renewing the species, in the vegetable creation, is performed entirely by her unerring power: and the fruit when it becomes fully ripened, drops off spontaneously without the hand of art to separate it. In the whole animal race this process is equally distant from disease (there are exceptions). Why then should the human species alone, her noblest production, undergo her unkindness and neglect in so material an object?'

It therefore seems appropriate to include as an example of White's writing this extract from his chapter on natural birth and fetal adaptation to extrauterine life.

Charles White of Manchester, 1728–1813.

'In the beginning of the labor I would be so far from confining my patient to any one position, that I would not even confine her to a single room, but would let her walk about from one apartment to another. Whenever a pain should oblige her to lie down I would take that opportunity of examination, that I might know whether the child was in a right position, and how fast the labor was advancing. During the whole time of her travail she ought to enjoy the fresh air; she should not be crowded with more friends or attendants that necessity required . . . Where the accoucheur is satisfied that the labor is natural, and that every thing is proceeding well, the patient should not be teized by attempting to hasten her delivery, nor even by too frequent examinations.

When the business is so far advanced that there is reason to believe the child will soon be born, it is in my opinion of great consequence that the woman should be in an horizontal position, and it will be most convenient if she lies upon her side with her back towards the practitioner . . . When the perineum begins to protrude, the pressure of a hand against that part will give great ease to the patient; the degree of pressure must be left to the judgement of the person employed, but if the pains are very forcing, it ought to be such as will prevent a too hasty delivery. If this caution be observed, and the patient be kept in an horizontal position, there will be no danger of a laceration of the perineum.

As soon as part of the head is produced . . . leave things to nature, and in general she performs her work the best, without assistance. After the patient has recovered herself a little, the pain will return, the shoulders will make their proper turns and be properly expelled . . . After a child is expelled in this gradual manner by the force of the woman's pains, the womb by degrees contracts itself . . . Where nature is very slow in relieving itself, assistance ought to be given, but not till it is seen how far she is able to do without it.

The common method of tying and cutting the navel string in the instant the child is born, is likewise one of those errors in practice that has nothing to plead in its favour but custom. Can it possibly be supposed that this important event, this great change which takes place in the lungs, the heart, and the liver, from the state of the foetus, kept alive by the umbilical chord, to that state when life cannot be carried on without respiration, whereby the lungs must be fully expanded with air, and the whole mass of blood instead of one fourth part be circulated through them, the ductus venosus, foramen ovale, ductus arteriosus, and the umbilical arteries and vein must all be closed, and the mode of circulation in the principal vessels entirely altered—Is it possible that this wonderful alteration in the human machine should be properly brought about in one instant of time, and at the will of a by-stander? Let us but leave the affair to nature, and watch her operations, and it will soon appear that she stands not in need of our feeble assistance, but will do the work herself, at a proper time, and in a better manner. In a few minutes the lungs will be gradually expanded, and the great alterations in the heart and blood vessels will take place. As soon as this is perfectly done, the circulation in the navel string will cease of itself, and then if it be cut no haemorrhage will ensue from either end; notwithstanding this, it will be always adviseable to tie it, as an haemorrhage might come on if the circulation should be quickened by the warmth of the cloaths and the bed. If the funis be cut immediately after the birth of the child, or before the pulsation in it ceases, that end next to the placenta will bleed about three or four ounces, and if that end next to the child was not tied it would in probability bleed to death. Whatever method be pursued it is better not to tie that end next to the placenta, for the more it is lessened by the blood being drained from it, the greater liberty is given to the uterus to contract.

By this rash, inconsiderate method of tying the navel string before the circulation in it is stopt, I doubt not but many children have been lost, many of their principal organs have been injured, and foundations laid for various disorders.

When the infant is removed, the secundines are sometimes found wholly expelled; sometimes the placenta is extruded from the womb and lying in the vagina, in which case it is to be handled gently, and with great care gradually brought away . . . Sometimes an interval of eight or ten minutes succeeds the birth of the child, when a pain coming on, the secundines will be easily extracted by gently pulling the navel string, and here an easy pressure upon the abdomen by assisting the uterus to contract will be of service. If the placenta is very large, a finger may be introduced to bring down one edge of it as soon as it is within reach. In this manner I have proceeded for several years, and during that period I can with satisfaction declare that in natural labors I have never had occasion for the manual extraction of the placenta; I have never left my patient till it came away, nor have I ever been detained a single hour by it . . .'

In 1790 Charles White resigned from the Manchester Infirmary after an argument with the Board of Management and founded the Manchester and Salford Lying-In Charity, which later became St Mary's Hospital for Women and Children. Finally, in 1813 after 'a long life of unremitting exertion of great and extensive usefulness' he died at the age of 84. St Mary's Hospital for Women and Children, Manchester celebrates its bicentenary this year.

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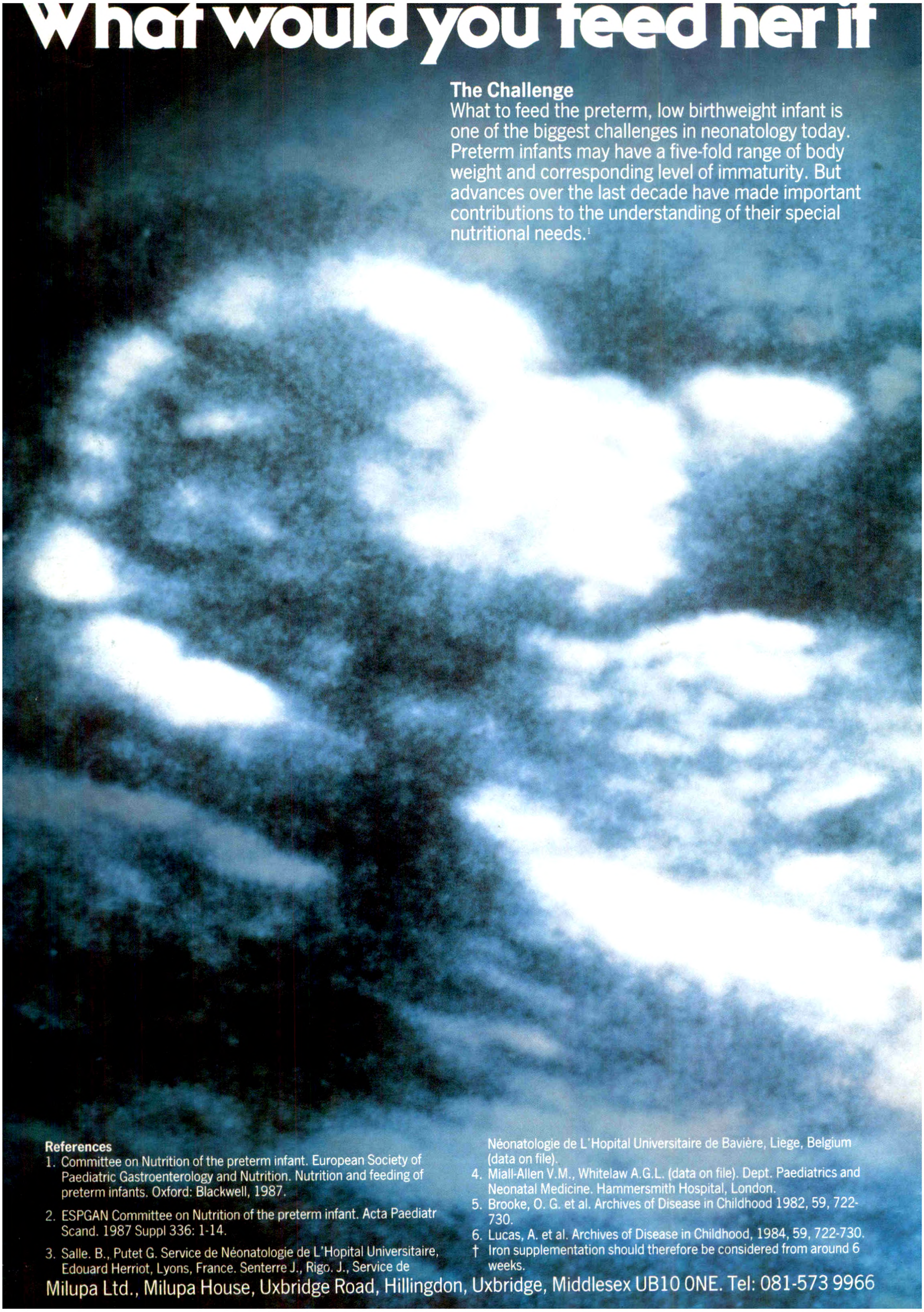

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† Iron supplementation should therefore be considered from around 6 weeks.

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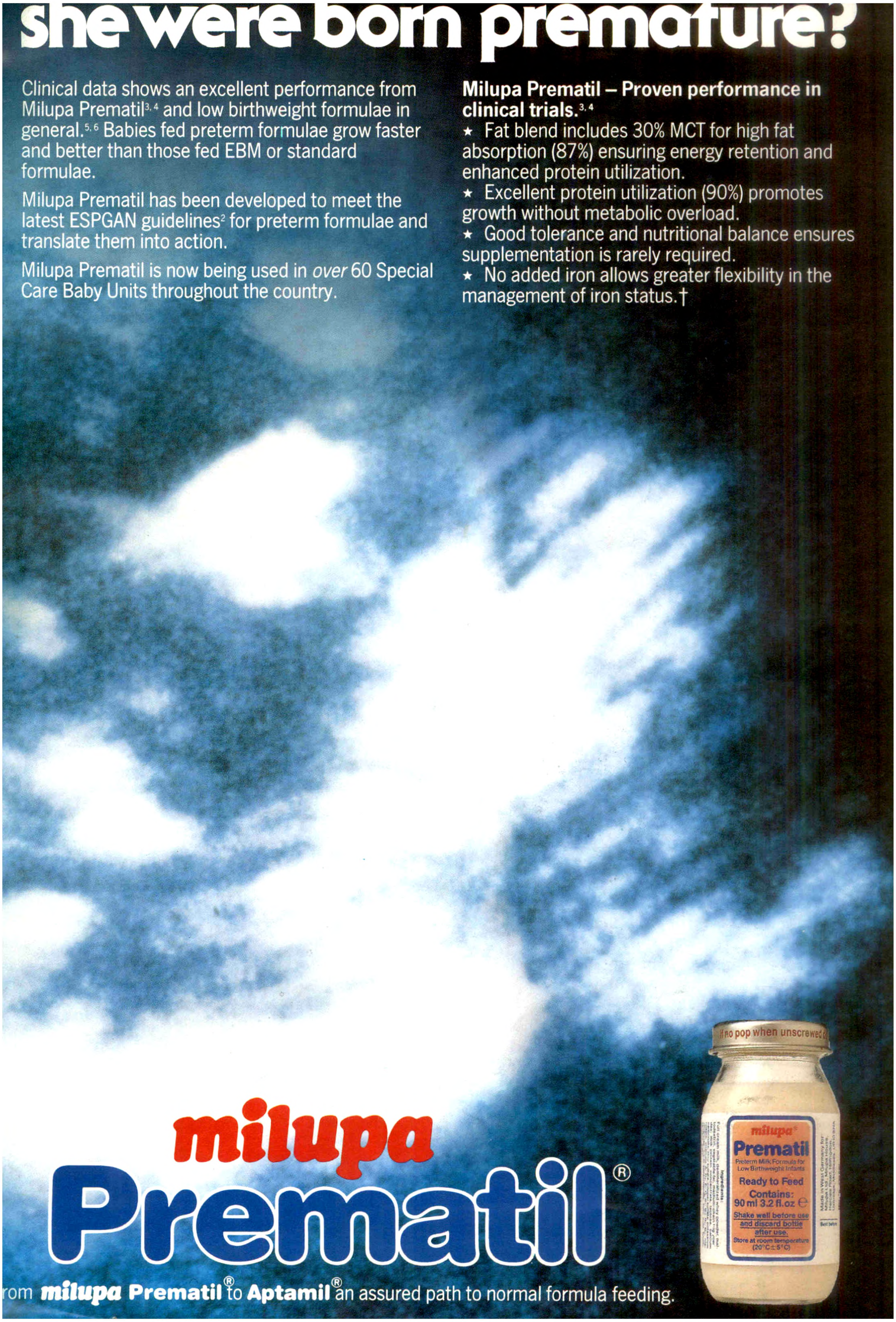
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Annotation

Respiratory problems and cystic adenomatoid malformation of lung

Congenital cystic disease of the lung accounts for about 25% of lung malformations, and is an uncommon but important cause of neonatal respiratory distress. Congenital cystic disease includes: (1) lobar emphysema, which tends to affect a single upper lobe, is due to the obstruction of a lobar bronchus, and is consequent upon an abnormality in the bronchial cartilage allowing bronchial collapse and distal overinflation. (2) Bronchogenic cysts, which are unilocular and lined with ciliated respiratory epithelium, and are usually adjacent to major bronchi but do not communicate with them. They probably represent a malformation developing at the time of closure of the primitive foregut. (3) Pulmonary lymphangiectasia, which is rare, usually bilateral, and is due to congenital cystic dilatations of the pulmonary lymphatics. (4) Congenital cystic adenomatoid malformation of the lung, which tends to affect the lower lobes, and is thought to be due to an excessive overgrowth of terminal bronchiolar structures (that is, a hamartoma), and is lined with ciliated columnar epithelium but without any systematic bronchial architecture present. There is a wide range, varying from lobes containing cysts with minimal or no adenomatosis (more common, and may result from an embryonic defect occurring before 49 days' gestation) to adenomatosis with infrequent cysts (rare, and probably due to a defect occurring before 26 days' gestation).

Presentation

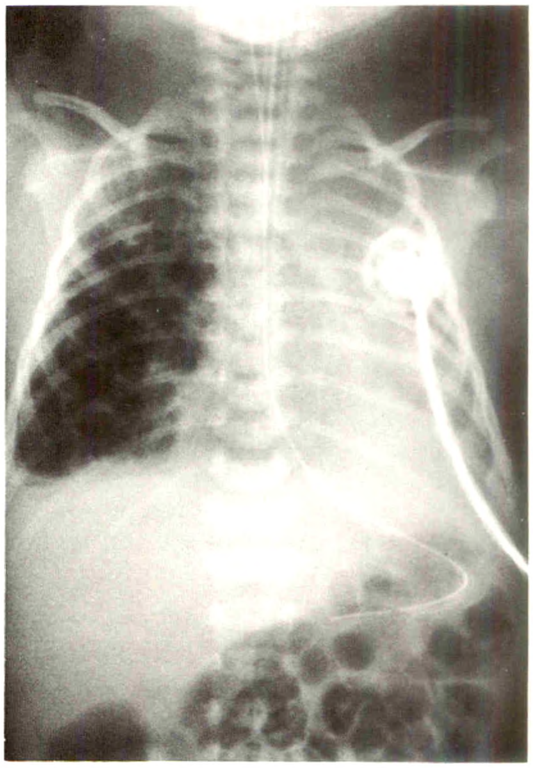
Patients with cystic adenomatoid malformation of the lung present with varying degrees of respiratory difficulty. Eighty percent of them present in the neonatal period with respiratory distress, often within hours of birth, and 14% are stillborn,[1] Only 10% present after the first year of life, and they do so because of recurrent respiratory infections.

Neonates with the tachypnoea and dyspnoea of respiratory distress require urgent investigation to identify the cause. Cardiac abnormalities should be considered, and a chest radiograph must be taken to differentiate between hyaline membrane disease, infection, or a congenital malformation (for example, congenital diaphragmatic hernia or congenital lung malformation).

Cystic spaces seen in the chest on radiography initially raise suspicion of a congenital diaphragmatic hernia, with loops of bowel containing gas in the chest, as this is the most common diagnosis. It is therefore essential that a chest radiograph is taken with a nasogastric tube in situ, with enough abdomen visible on the radiograph to be able to see the full length of the tube, so that oesophageal displacement suggesting mediastinal shift, the position of the stomach, and the site of intestinal gas can be identified. However, cystic adenomatoid malformation can be differentiated on radiography by the presence of a normally situated stomach and intestine within the abdomen.

Cystic spaces in the chest can occur in association with infection, for example post staphylococcal pneumatocoeles. This diagnosis can be identified with the help of a reliable history and serial radiography.

The correct identification of cystic spaces in the chest



Radiograph of chest and upper abdomen demonstrating multiple cystic spaces in the right lung with mediastinal shift to the left, with a nasogastric tube entering a normally situated stomach with normal distribution of bowel gas and liver shadow.

being due to cystic adenomatoid malformation of the lung requires awareness of the diagnosis and exclusion of other possible causes.

Treatment

Immediate management of a patient with cystic adenomatoid malformation is by appropriate treatment of the respiratory distress. The cystic spaces within the abnormal lobe will continue to increase in size as they aerate further. Morbidity is due to pressure of the expanded affected lobe onto the ipsilateral normal lobes and associated mediastinal shift with compression of the contralateral normal lung. Delay in diagnosis and hence appropriate treatment increases pressure symptoms, and clinical signs worsen. Respiratory distress may become severe and the baby may require supportive ventilation. The cysts are very prone to secondary infection, and this requires recognition and appropriate treatment.

Definitive treatment of cystic adenomatoid malformation of the lung is by thoracotomy and excision of the affected lobe or lobes. It is therefore essential to differentiate between congenital diaphragmatic hernia and cystic adenomatoid malformation of lung preoperatively in order to avoid an unnecessary laparotomy, which has happened, even in our own series.

In the last 36 years (1954–89) at this hospital, 16 neonates have undergone operative treatment for congenital cystic adenomatoid malformation of the lung. Three of these presented in September 1986, but no predisposing factors for this clustering have been identified. One of these patients was transferred to us with the diagnosis of congenital diaphragmatic hernia, but a repeat chest and abdominal radiograph (figure) with a nasogastric tube in situ enabled us to make the correct diagnosis before operation.

All these 16 patients (nine boys and seven girls) were born at term and presented with respiratory distress, nine within hours of birth, two in the first week, and the remaining five within four weeks. The patients were all treated surgically. Two patients early in the series had an initial inappropriate laparotomy, followed by a thoracotomy and lobectomy, and the other 14 patients had thoracotomy and excision of the affected lobe—the lower lobes being the most common (left n=8, right n=4). The left upper lobe was affected in one patient, the right middle lobe in one patient, and one died who was found to have had total involvement of both lungs. One patient proved subsequently to have cystic staphylococcal pneumonia.

These facts are similar to other published series.[1 2]

Antenatal diagnosis

Diagnosis has been reported antenatally,[3–6] and cystic adenomatoid malformation is one of the differential diagnoses of a mass in the chest seen on an antenatal ultrasound scan. Location of the stomach will particularly help in differentiating between cystic adenomatoid malformation and congenital diaphragmatic hernia.

As with all antenatal diagnoses of congenital malformations, it is important as it allows time for preparation and advice to the parents, and better planning of the place of delivery and subsequent management and treatment.

Prognosis

Neonates tolerate lobectomy extremely well, and follow up of our three recent patients with isotope studies shows that the remaining lung grows normally and expands to fill the thoracic cavity (unlike patients with repaired congenital diaphragmatic hernia where many of the lungs have a degree of hypoplasia). Clinically the lung functions well, although respiratory function tests may be expected to show a diminution of function.

Conclusions

Congenital cystic adenomatoid malformation of the lung can present with respiratory distress in the newborn. An awareness of the diagnosis is essential for all physicians involved in the care of neonates. All babies with cystic appearances on their chest radiography should have a radio-opaque nasogastric tube passed and a radiograph taken that includes the chest and abdomen in order to localise the oesophagus, stomach, and bowel gas to avoid mistaking the condition for a diaphragmatic hernia and hence the possibility of an inappropriate laparotomy.

The passage of time increases the problems caused by pressure from the aeration of the cystic lung with mediastinal shift making this condition a surgical emergency, and urgent referral should be made to a regional paediatric surgical centre.

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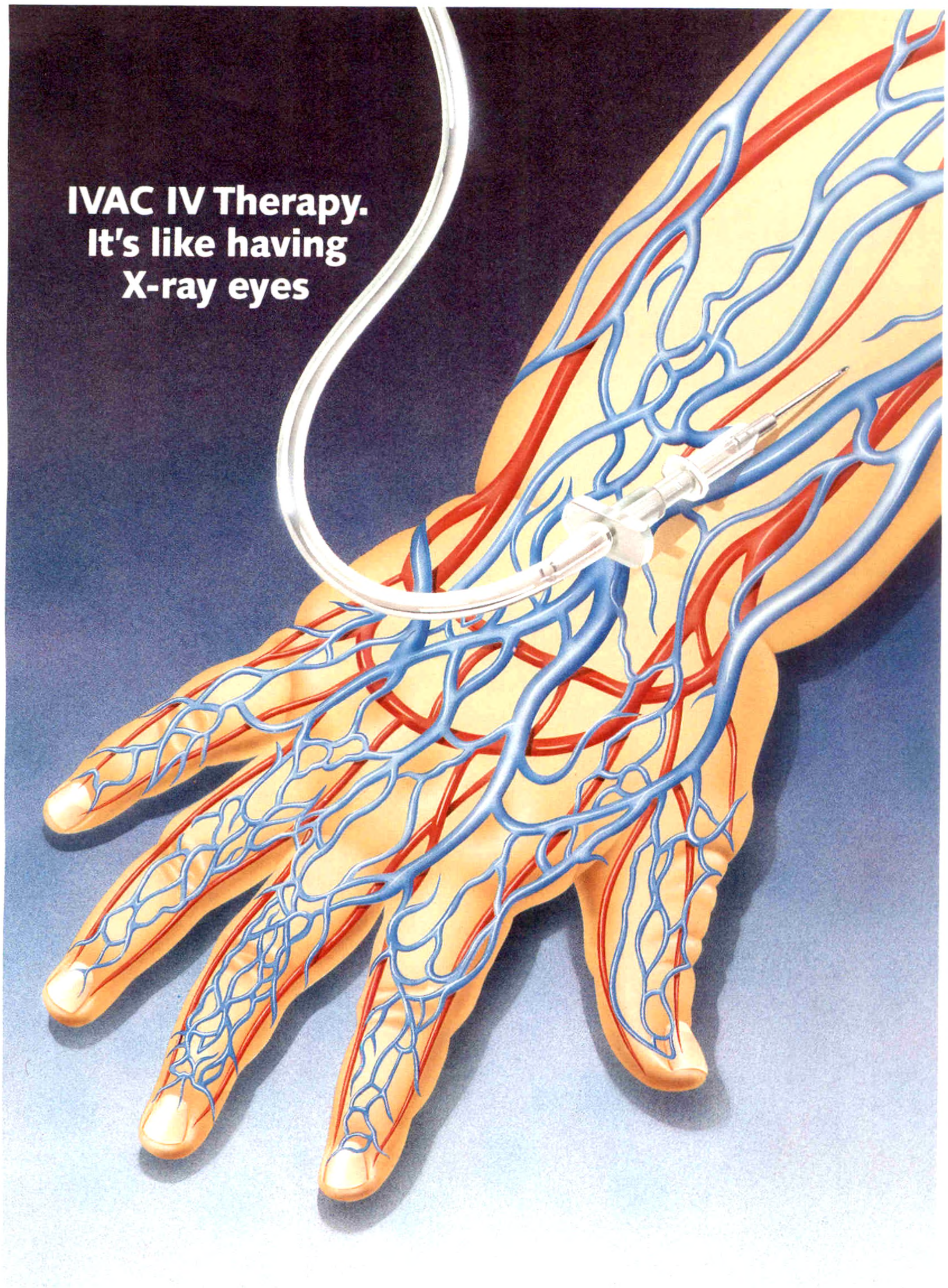

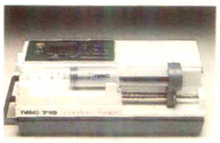

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ORIGINAL ARTICLES

Application of intragenic DNA probes in prenatal screening for retinoblastoma gene carriers in the United Kingdom

Z O Onadim, C D Mitchell, P C Rutland, B G Buckle, M Jay, J L Hungerford, K Harper, J K Cowell

Abstract

Restriction fragment length polymorphisms (RFLPs) in 55 families affected by retinoblastoma have been studied using recombinant DNA probes derived from within the retinoblastoma predisposition gene. Only six families were uninformative for any of the DNA polymorphisms. The remaining 49 families can be offered prenatal screening. No obligate recombinations between any of the polymorphic loci and the retinoblastoma phenotype were observed. Four previously unknown cases of non-penetrance were identified. Prenatal testing for the inheritance of mutant alleles was performed in two cases and perinatal screening in two additional cases. One fetus inherited the normal allele from the affected parent and is therefore not at risk of retinoblastoma; the second fetus inherited the mutant allele and will require frequent screening for early detection of retinoblastoma. Both perinatal tests showed the absence of the mutant allele.

Retinoblastoma, although rare, is the most common ophthalmic malignancy in childhood. It has been the focus of a large amount of research, because in about one third of cases the predisposition to cancer is dominantly inherited from affected parents.[1 2] Early detection of retinoblastoma ensures a better prognosis, both for life and for vision. Until recently the only available method of early detection was regular full ophthalmological assessment under anaesthetic. In practice this means that all children at risk of retinoblastoma on clinical grounds are screened. As the retinoblastoma mutation is not fully penetrant (not all subjects carrying the dominant mutant gene show the mutant phenotype) and the disease is able to 'skip' a generation, even children of unaffected subjects from within an affected family must be considered 'at risk' as well as those of apparently sporadic cases. Thus there is considerable investment in a small number of patients of whom less than half ultimately develop tumours. In addition, many patients with retinoblastoma have elected not to have children because of the risk of an offspring being similarly affected. The ability to detect the inheritance of normal or mutant retinoblastoma alleles (one of a series of possible alternative forms of a given gene differing in DNA sequence) would allow clinical screening procedures to focus on those patients at high risk of developing retinoblastoma, and make pregnancy, with prenatal testing and termination of predisposed fetuses, an option for those families not wanting to have an affected child.

The discovery that the esterase-D gene lay in chromosome band 13q14[3] meant that a protein polymorphism (the existence of two or more genetically different classes in the same interbreeding population) for that enzyme could be used for 'gene tracking' in families with retinoblastoma.[4] Although close linkage was firmly established,[5–8] the low incidence of heterozygotes in the population made it impractical to apply this technique widely. Randomly isolated DNA sequences, which had been mapped around the 13q14 region, and which recognised restriction fragment length polymorphisms (RFLPs—variations occurring within a species in the length of DNA fragments generated by a specific endonuclease) were used for prenatal screening.[9 10] Succes was, however, limited because of recombination (the occurrence of progeny with combinations of genes other than those that occurred in the parents as a result of independent assortment or crossing over) between these markers and the retinoblastoma locus.[11] In 1986 Friend *et al* isolated a candidate retinoblastoma gene that showed frequent structural abnormalities in retinoblastoma tumour cells.[12] Mutations of this gene have now been found in somatic cells of predisposed subjects.[13–14] It is not possible to recognise RFLPs directly using this complementary DNA (cDNA), but recently Wiggs *et al* isolated a series of DNA probes from within the genomic sequence that recognise high frequency RFLPs.[16] We have investigated the segregation of alleles detected by these sequences in a large series of families with retinoblastoma and report on their application to family linkage studies.

Subjects and methods

Most families were identified in the ophthalmic oncology clinics at St Bartholomew's Hospital and Moorfields Eye Hospital, although some were submitted from other regional centres. In each case the diagnoses of affected individuals has been confirmed by an experienced ophthalmologist (JLH). Blood samples were collected from as many relevant family members as possible and stored at −80°C until required. For many of the key family members lymphoblastoid cell lines were also generated. Prenatal samples were obtained either by chorionic villus sampling or by amniocentesis.

DNA was prepared from white blood cells, lymphoblastoid cell lines, chorionic villi, or cultured fetal fibroblasts using standard methods.[17] Between 2–5 μg DNA from each

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member of the family was digested with the appropriate restriction endonuclease, size fractionated by electrophoresis through 1% agarose gels, and transferred to nylon membranes (Hybond-N) as described by Southern.[18] All DNA probes were labelled to high specific activity by the oligoprimer extension method.[19] Prehybridisation and hybridisation were carried out in quadruple strength 0·15M sodium chloride and 0·015M sodium citrate (SSC) sonicated boiled salmon sperm, 10 µg/ml polyadenylic acid and 0·1% sodium dodecyl sulphate (SDS) at 65°C for 16 hours. Filters were then washed at 65°C for two washes of 15 minutes each in double strength SSC/0·1% SDS, and then exposed to Kodak XAR-5 autoradiographic film with intensifying screens for 16–72 hours.

Family pedigree data were prepared for analysis using the progam LINKSYS,[20] and log of the odds scores were calculated using the program LIPED 6.[21] Assuming that the frequency of new gene mutations gamete is $1{\cdot}5\times10^{-5}$, data were analysed for a penetrance (the proportion of subjects in a specified group that show the expected phenotype under a set of environmental conditions) of 0·9.

Results

PATTERN OF INHERITANCE

In our series there were 17 families in which subjects had either an affected parent or sibling, and also an affected child. Three of these 29 subjects were not themselves affected and ophthalmological examination of their retinas failed to detect evidence of regressed tumours. From these observations we assess the penetrance of the retinoblastoma gene as 90% and we have used these figures to calculate the log of the odds scores.

The inheritance of the retinoblastoma phenotype follows the normal pattern of autosomal dominant inheritance in most families (fig 1). Family RBF-12 is unusual in that each of the affected members has only a unifocal tumour or evidence of spontaneous regression (fig 2). Phenotypic analysis indicates that there is an apparently unaffected transmitting member (II.7). Unfortunately this subject was not available for ophthalmological examination; it is possible he also had regressed tumours, especially as evidence of incomplete penetrance in another member of the family was detected using molecular probes (fig 2). The log of the odds score for this family was −0·03 at θ=0 and 90% penetrance and 0·178 at θ=0 and a penetrance of 80%. θ Represents the proportion of recombinants out of all the opportunities for recombination. Assessing the log of the odds score, assuming 90% penetrance, the maximum value of 0·96 was obtained at θ=0·2 (table 1). In family RBF-04 three affected siblings were

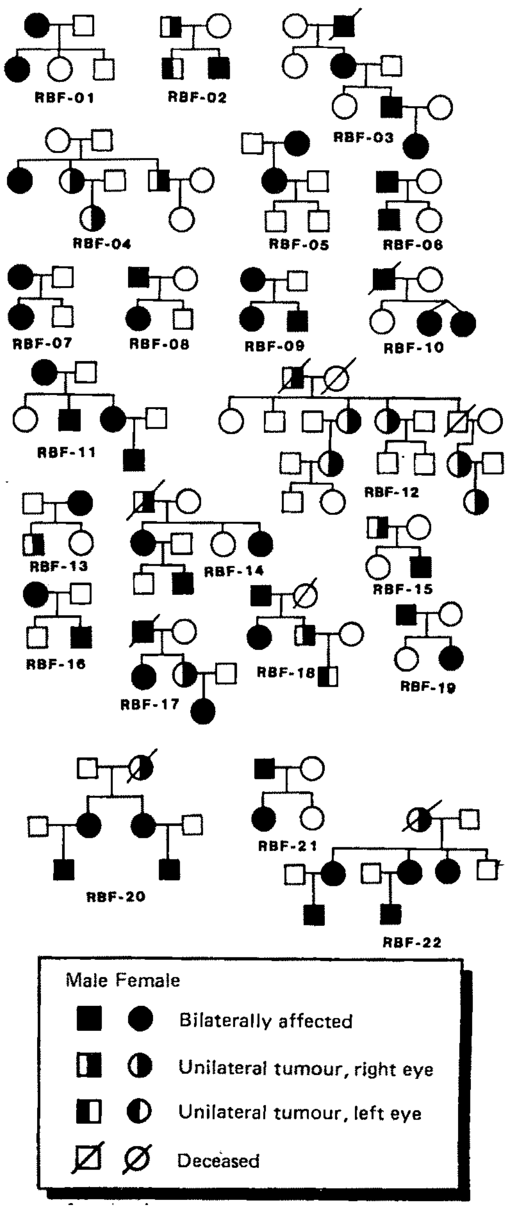

Figure 1 Structure of family pedigrees used in linkage analysis with the intragenic, polymorphic DNA gene probes.

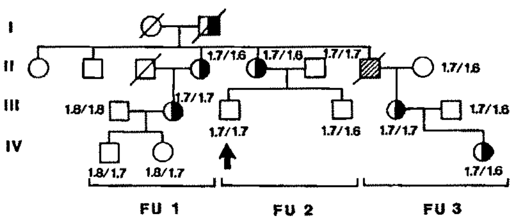

Figure 2 Segregation of RS2·0 alleles in family RBF-12. The Rb phenotype is segregating with the 1·7 allele. In family unit (FU) 1 the affected subject (III.2) is homozygous so it is not possible to determine whether their children are carriers (IV.1, IV.2). In family unit 2 both children are unaffected but III.3 (arrow) has received the 1·7 Kb allele from his father and therefore must be a gene carrier. In family unit 3 both affected members have only retinal scarring as the sign of the retinoblastoma gene and the grandfather (II.7) was unavailable for analysis.

Table 1 Log of the odds scores at different values of θ for the two families that showed evidence of incomplete penetrance

Families	*Score*										
	0·0	*0·001*	*0·005*	*0·1*	*0·15*	*0·2*	*0·25*	*0·3*	*0·35*	*0·4*	*0·45*
RBF-04	0·048	0·048	0·056	0·058	0·055	0·048	0·039	0·029	0·019	0·009	0·003
RBF-12	−0·030	−0·028	0·037	0·072	0·090	0·096	0·092	0·079	0·058	0·033	0·010
Total	0·018	0·02	0·093	0·130	0·145	0·144	0·131	0·108	0·068	0·042	0·013

born to unaffected parents. In this family it was not possible to determine whether one of the parents was a true case of incomplete penetrance or whether one or the other was a gonadal mosaic for the mutation. The maximum log of the odds score in this family assuming a penetrance of 90% was 0·058 at $\theta=0{\cdot}1$. The log of the odds scores for 20 informative families were calculated and are shown in table 2. The cumulative scores, including that derived from family RBF-12, was 5·947 at $\theta=0$ and 90% penetrance.

GENE TRACKING WITH INTRAGENIC PROBES

Five unique DNA sequence probes from within the genomic sequence of the 4·7R (RB1) gene were used in this study (table 3).[16] Each probe recognises RFLPs that occur sufficiently frequently to be of value in linkage analysis.

Fifty five families were analysed, and they form two groups. The first (n=22) contained at least two children and had affected family members in at least two generations, in most cases allowing log of the odds scores to be calculated. The pedigrees of these families are shown in fig 1. In the second group of families (n=33), either there was only one affected child, or not all family members were available for testing, but we were able to establish the allele with which the retinoblastoma phenotype was segregating in order to offer prenatal screening in the future. All the families were selected because prenatal screening could be offered to at least one family member if requested.

Table 2 Log of the odds scores from informative retinoblastoma families

Families	*Penetrance 90%*
RBF-02	0·301
RBF-03	0·260
RBF-04	0·048
RBF-05	0·223
RBF-06	0·260
RBF-07	0·260
RBF-08	0·260
RBF-09	0·301
RBF-10	0·561
RBF-11	0·862
RBF-12	−0·030
RBF-13	0·260
RBF-14	0·422
RBF-15	0·260
RBF-16	0·260
RBF-17	0·125
RBF-18	0·085
RBF-19	0·260
RBF-20	0·204
RBF-21	0·561
RBF-22	0·204
Total	5·947

Allele frequencies for the five probes in the British population were determined from an analysis of unrelated subjects within the families studied, and are listed in table 3. The most highly informative probe was RS2·0 which, following digestion of the genomic DNA with the RSA1 restriction enzyme, identified a variable number tandem repeat (VNTR—an aberration in which two identical chromosomal segments lie one behind the other. The order of the repeat unit in each segment is the same). The polymorphism identified by RS2·0 detected a VNTR of roughly 50 base pairs, with eight alleles so far detected.[16] To separate these alleles, DNA (2–3 μg) is run overnight in an 0·4 cm thick, 1·2% agarose gel until lambda markers below 1·0 Kb in size have migrated off the bottom of a 20 cm gel. Under these conditions it is usually possible to distinguish betweeen alleles differing in size by only 50 base pairs. Variations in gel thickness, however, conditions which result in non-homogeneity of the gel, or local heat gradients during electrophoresis can distort the migration rate of the DNA restriction fragments. Thus it is difficult to establish the sizes of alleles and compare them among gels. This almost certainly accounts for the discrepancy between our estimate of allele frequencies and those reported by Wiggs *et al.*[16] Within a single gel, however, the mendelian inheritance can easily be followed (fig 3). In some families only key members were analysed with this probe. If they were heterozygotes the analysis was extended to the remaining family members. Homozygotes were not investigated further but were analysed with the other probes, which identify less frequent RFLPs (table 4). The second most informative probe was PRO·6 and the third was M1·8 (fig 4), the two other less informative probes being used only if required.

Six families (11%) were uninformative for all the probes used so far and cannot therefore be offered prenatal screening. Of the remainder,

Table 3 Summary of frequencies with which alleles occurred in the population of the United Kingdom intragenic retinoblastoma probes

Probe	*Enzyme*	*No of individuals*	*Size of allele (kb)*	*Percentage frequency of allele in population*	*No of families*	*No (%) that were informative*
RS2·0	RSA1	107	2·0	0·09	55	40 (73)
			1·95	0·10		
			1·9	0·23		
			1·85	0·13		
			1·8	0·28		
			1·75	0·02		
			1·7	0·06		
			1·6	0·05		
			1·5	0·04		
PRO·6	XbaI	132	7·8	0·48	45	19 (42)
			6·5	0·52		
M1·8	BamHI	66	4·5	0·34	30	13 (39)
			2·3/2·2	0·66		
RO·6	Tth111	64	4·95	0·60	14	4 (29)
			4·35	0·40		
HSO·5	Kpn 1	43	12·0	0·93	10	0
			8·0	0·07		

Table 4 Families with retinoblastoma in which a given RFLP was informative in a particular kindred

Family	*Probes*				
	p68RS2·0	*p88R0·6*	*p123M1·8*	*p95HS0·5*	*p35R0·6*
RBF-01	Yes	No	No	Yes	Not tested
RBF-02	Yes	No	No	Yes	No
RBF-03	No	Yes	No	No	No
RBF-04	Yes	No	No	No	No
RBF-05	Yes	Yes	Not tested	Not tested	Not tested
RBF-06	Yes	No	Yes	Yes	No
RBF-07	Yes	Not tested	No	Not tested	Not tested
RBF-08	Yes	Yes	Yes	Yes	No
RBF-09	Yes	No	Yes	No	Not tested
RBF-10	Yes	Not tested	Not tested	Not tested	Not tested
RBF-11	No	No	No	No	Not tested
RBF-12	Yes	Yes	Yes	No	Not tested
RBF-13	Yes	Yes	Yes	No	Not tested
RBF-14	Yes	No	No	No	No
RBF-15	Yes	No	Yes	Yes	Not tested
RBF-16	Yes	No	Yes	No	Not tested
RBF-17	Yes	No	No	No	No
RBF-18	Yes	Yes	Yes	No	Not tested
RBF-19	No	No	No	No	Yes
RBF-20	Yes	Yes	Not tested	Not tested	Not tested
RBF-21	Yes	No	Yes	Not tested	Yes
RBF-22	Yes	No	No	Not tested	No

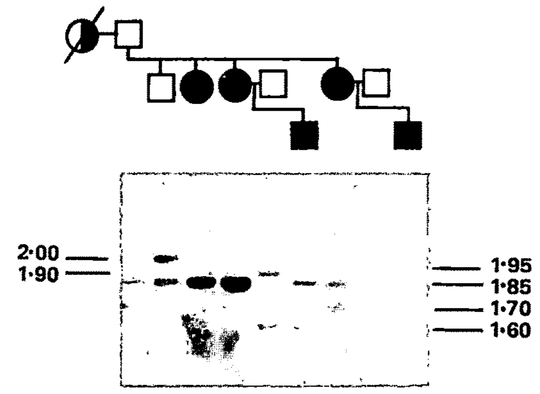

Figure 3 Linkage analysis in family RBF-22 using the RS2·0 intragenic Rb probe. DNA from family members shown in the pedigree (fig 1) was digested with RSAI and six different alleles ranging in size between 2·0–1·6 Kb were detected. In this family the mutant gene is segregating with the 1·85 Kb allele. The genotype of the subject represented in lane 9, showing only weak hybridisation, was 1·85/1·60.

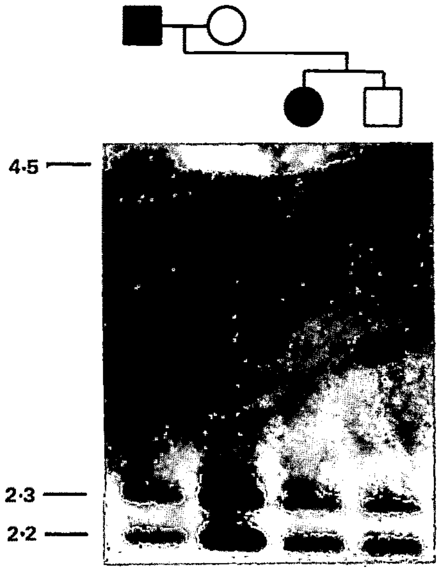

Figure 4 Linkage analysis in family RBF-8 using the M1·8 intragenic Rb probe. Individual alleles are either 4·5 Kb long or a doublet, 2·3 and 2·2 Kb long. In this case the disease phenotype is segregating with the lower allele.

73% were informative with RS2·0 alone. Of the 16 families not informative for RS2·0, 11 (69%) were informative for PRO·6.

In several cases, though the transmitting subject was heterozygous, the children became homozygous for a particular probe. In these cases the analysis was extended so that informative probes were also available for affected subjects in the second generation.

PRENATAL SCREENING

The possibility of screening for carriers has been discussed with each family. So far two infants have been screened perinatally and in both cases it was possible to say that they were not carriers. Two families have taken advantage of prenatal screening. In the first (RBF-14), DNA was obtained by chorionic villus sampling,[22] and in the second (RBF-01) from cultured amniotic fluid cells obtained for chromosome analysis because of advanced maternal age. The analysis of family RBF-01 with the RS2·0 probe is shown in fig 5. The affected and unaffected children have both inherited the same maternal allele, suggesting that the unaffected daughter apparently carries the mutant allele, as does the fetus. Alternatively, a recombination event within the RB1 locus in the affected daughter, proximal to the RS2·0 site, may have occurred. Because available flanking markers were homozygous in the transmitting parent, however, it is not possible to exclude recombination. This family was not included in the log of the odds score calcula-

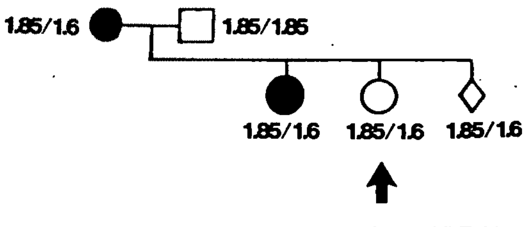

Figure 5 Segregation of RS2·0 alleles in family RBF-01. The retinoblastoma phenotype is segregating with the 1·6 Kb allele, which is also present in the unaffected 8 year old daughter (arrow) as well as in the amniotic cell from the fetus (◇).

tions. No obligate recombination was detected among markers or among the markers and the retinoblastoma phenotype in any of the other families. The region of the genome covered by the retinoblastoma gene is approximately 200 kb. This distance represents about 0·2 centimorgan (cM) and therefore recombination frequency should be low.

Discussion

Using five intragenic, unique sequence DNA probes for the retinoblastoma locus, 90% of families were informative for prenatal screening. As we have used probes from within the retinoblastoma gene itself, recombination among markers is unlikely. Nevertheless in the absence of mutation specific probes, recombination between marker and mutation cannot be excluded. Data from this series and from that of Wiggs *et al*[16] give a cumulative log of the odds score of 14·55 at $\theta=0$, assuming a penetrance of 0·9.

Not all subjects who inherit a mutant retinoblastoma gene develop the tumour, a phenomenon referred to as incomplete penetrance. In the inherited form of retinoblastoma it is believed that non-penetrance arises because the predisposed retinoblasts do not suffer the second mutation that would result in the development of malignancy. Vogel reviewed all published pedigrees up to 1979 and calculated that penetrance was between 0·996 to 0·927, with a higher frequency in offspring of bilaterally affected parents.[1] Our calculation of penetrance (clinically defined) is in agreement with these figures, though selection bias is possible as the families in this series were mostly from a single referral hospital. Using RFLP analysis we have identified apparently unaffected gene carriers. Presumably these subjects remain at risk of other tumours associated with retinoblastoma, and their offspring are at risk of retinoblastoma; they will now receive appropriate genetic counselling. Conversely, the (unaffected) subjects shown not to be carriers of the predisposition allele may be reassured that they do not carry the mutant allele.

Incomplete penetrance can also be the result of inheritance of a balanced translocation where the unbalanced form gives rise to tumour predisposition[23] or missed diagnosis where apparently unaffected gene carriers show evidence of regressed tumours or retinomas.[24] Excluding family RBF-01, where the issue of penetrance has still to be resolved, two other families showed evidence of unaffected gene carriers. In one family, for whom gene tracking using the intragenic probes was not possible, the incomplete penetrance was completely predictable from phenotype analysis, the remainder of the affected subjects having the full range of phenotypic expression of the retinoblastoma gene. In family RBF-12, however, no subject has had bilateral tumours, and in one family nucleus affected members bore only retinal scarring. This family is reminiscent of that reported by Connolly *et al*.[25] It is possible that in this family the phenotype may result from specific mutations resulting in non-penetrance, rather than contributing to that part of the Poisson distribution described by Knudson, where the second mutation has not occurred.[26] The availability of the retinoblastoma gene sequence may answer this question in the future. Within this family we have detected unaffected gene carriers who do not have retinal scarring. Cytogenetic analysis (JK Cowell, unpublished observations) failed to show any serious structural abnormality of chromosome 13 in affected family members, and their esterase-D gene concentrations were normal,[27] precluding any large deletion in 13q14.

There are ethical issues to consider in the prenatal diagnosis of a disease such as retinoblastoma, which is rarely lethal and has no effect on mental ability. Although the vision of some patients who are treated early for single tumours may not be greatly affected, in some cases enucleation (which may be bilateral) is necessary. This operation is disfiguring, and many parents have expressed an interest in screening. Even though the child will survive the primary tumour, 5–15% will develop second tumours in adolescence, usually soft tissue sarcomas or osteosarcomas.[28 29] In family RBF-14, the first to opt for prenatal screening, many of the members had bilateral disease, and were blind or partly sighted. It was their belief that in the event of a fetus inheriting a mutant allele, termination of pregnancy was acceptable. In the second family, RBF-01, termination was not acceptable, particularly as an unaffected previous child could have been the result of non-penetrance or a recombination between the probe and the predisposing mutation. In both cases the validation of the results awaits the passage of time.

In linkage studies such as this it is essential that the affected parent is heterozygous for at least one of the polymorphisms. Such is the variety of probes now available that most families are informative. Using standard linkage analysis prenatal screening can be offered to 90% of families, but new probes are still required for the remainder. The discovery of a VNTR, based on a four base pair repeat in the region adjacent to exon 20,[30] should be an important addition to the screening armoury. There are between 10 and 24 different alleles in this system and it is estimated that 97% of subjects will be heterozygous at this locus. Although the techniques used for this analysis are more complex than in conventional agarose gel electrophoresis, specialist genetic laboratories should be able to apply this procedure to prenatal screening. The ultimate goal is to determine whether each newly diagnosed case of retinoblastoma is a carrier; there is likely to be a whole range of mutations in different families. Though the whole retinoblastoma gene is large, the 27 exons occupy only approximately 5 Kb. Polymerase chain reaction amplification, and sequencing of each exon and their flanking regions offers a realistic possibility in the near future of providing unequivocal evidence for specific (gene) mutations in individual families. If this technique can be applied to the analysis of apparently sporadic cases it should be possible to detect carriers in the absence of a family history.

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Fetofetal transfusion syndrome: do the neonatal criteria apply in utero?

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Abstract

Thirteen fetuses (five twin, one triplet) were compromised by fetofetal transfusion syndrome in six pregnancies, five in the mid trimester, and one in the third trimester. This diagnosis, which was suspected because of ultrasound findings of discordant growth, discordant amniotic fluid volumes, concordant external genitalia, and monochorial placentation, was confirmed postnatally in each. Nine fetuses underwent blood sampling to aid diagnosis and assessment of fetal wellbeing. In contrast to fetofetal transfusion syndrome investigated postnatally, a difference in haemoglobin concentration of 50 g/l or more in utero was found in only one pregnancy, which was near term, although all had fetal erythroblastaemia and a difference in weight of 20% or more. In vivo confirmation of shared circulation was achieved in two pregnancies by transfusing adult Rh negative red cells into the smaller fetus and then detecting them by Kleihauer testing in blood aspirated from the larger. Invasive procedures also yielded information on fetal blood gas measurements (acidaemia in four and hypoxaemia in six) and amniotic pressure (raised in two). We suggest that comparison of haemoglobin concentrations is inaccurate in fetofetal transfusion syndrome in utero, the diagnosis of which may necessitate detection of a shared circulation using a marker such as adult red cells.

Although vascular communications are found in the placentas of almost all monochorial twin pregnancies, clinical signs of twin to twin transfusion occur in only 4–26%.[1 2] The pathophysiology of this condition, known as fetofetal transfusion syndrome, is poorly understood. In general however, the donor twin becomes anaemic, growth is retarded, and it develops oligohydramnios, whereas the recipient develops hydrops, cardiomegaly, and polyhydramnios.[3]

Most reported cases have been diagnosed postnatally or at necropsy. The standard neonatal criteria comprise a difference in cord haemoglobin concentrations of 50 g/l or more, and a difference in birth weights of 20% or more,[4–6] although the latter criterion is not necessarily present in the acute form of fetofetal transfusion syndrome that occurs in labour.[7] Certain antenatal ultrasound appearances are highly suggestive of fetofetal transfusion syndrome,[8–11] principally discrepant growth, and amniotic fluid volumes between monochorionic diamnionic twins.[8 10 11] Discordant growth, however, is a common complication of twin pregnancies, and causes other than fetofetal transfusion syndrome exist for discordant amniotic fluid volume. Signs of hydrops fetalis on ultrasound scan are occasionally found in the recipient,[10 11] rarely in the donor,[4] and exceptionally in both.[12] As all the above ultrasound features are non-specific, more accurate methods of antenatal diagnosis are needed if its associated high perinatal mortality rate of 40–81% is to be reduced.[8–10 13]

Pregnancies complicated by fetofetal transfusion syndrome will increasingly be evaluated in utero now that some of its signs—for example, intrauterine growth retardation, hydrops, and abnormal amniotic fluid volume, have become accepted indications for fetal blood sampling. Although precise criteria exist for the postnatal diagnosis of fetofetal transfusion syndrome, none have as yet been defined for its diagnosis in utero. We describe our experience of detailed fetal assessment in fetofetal transfusion syndrome using ultrasound scanning and fetal blood sampling.

Patients and methods

Among multiple pregnancies referred over a three year period for investigation of discordant fetal growth, the diagnosis of fetofetal transfusion syndrome was strongly suspected in six (five twin, one triplet) because of ultrasonographic findings of discrepant amniotic fluid volume, concordant external genitalia, and a monochorionic placenta, in addition to discordant intrauterine growth retardation. Gestational ages ranged from 17–36 weeks, with only one patient beyond 27 weeks.

Initial assessment comprised detailed ultrasound scanning, examining in particular the site of the placenta, the thickness of the septum,[14] the depth of the amniotic fluid pools, and the presence of hydrops. In four cases, pulsed Doppler studies of umbilical arterial flow velocity waveforms were undertaken (Acuson, Mountain View, California, USA). The indications for fetal blood sampling were the investigation (karyotype and blood gas analysis) of severe intrauterine growth retardation in six, of hydrops in three, and of polyhydramnios in four, and the exclusion of coagulopathy in one twin after the intrauterine death of its monochorial sibling two to three weeks before.[9 15 16] Additional reasons for fetal blood sampling were in all cases to assess fetal wellbeing and to attempt to confirm the diagnosis by detecting differences in haemoglobin concentrations. All patients gave written informed consent. Fetal blood sampling was carried out with a 20 gauge needle inserted under ultrasound guidance. The site of sampling was the placental cord insertion in five, the intrahepatic vein in one, and a free loop of cord in three. Gross polyhydramnios precluded fetal blood sampling in the other fetus(es) in cases 3 and 6.

The purity and fetal source of samples was checked at the time by separate red cell volume distributions on a particle size analyser (Coulter Channelyzer C-256, Coulter Electronics) and later confirmed by the Kleihauer test.[17] The packed cell volume and haemoglobin concentration in fetal blood were determined by a Coulter S Plus Counter. Nucleated red cells and reticulocyte counts were calculated from ratios obtained from blood films, which in turn were applied to results from the automated cell counter as described previously.[18] Blood gas analysis was performed on 250 μl of fetal blood collected into heparinised capillary tubes.[19] Published normal ranges were used for haemoglobin,[20] packed cell volume,[21] erythroblasts,[22] and blood gases.[23] Karyotypes were determined in fetal blood after short term fibroblast culture.

To document the presence of placental vascular anastomoses in vivo, adult blood (group O Rh negative and seronegative for cytomegalovirus was transfused into two donor fetuses. Fetal blood samples were then taken from their presumed recipient sibling to determine, using the Kleihauer technique, the passage of transfused adult red cells from one twin to the other.

The needle used for taking the sample of fetal blood was also used to provide access to the amniotic cavity. Four patients with gross polyhydramnios underwent therapeutic drainage of amniotic fluid to reduce amniotic pressure.[24 25] Amnioinfusion was carried out in one fetus to allow a thin septum to be seen; it had previously not been visible, but was suspected because of fetal immobility.[14]

Necropsy was performed by one of two perinatal pathologists in all but one case of fetal loss, in which consent was withheld. Postpartum evaluation of the configuration of placental membranes confirmed monochorionic diamniotic or triamniotic placentas in all. Meticulous injection studies were not performed, although shared areas on the chorionic surface were evaluated macroscopically. Pathological extramedullary haemopoiesis was defined as histological evidence of increased hepatic haemopoiesis out of proportion to that seen in normal fetuses of similar gestational age.

For reasons of clarity, sibling fetuses are denoted by the letters A to C, with the recipient fetus in each pregnancy referred to as fetus A.

Results

Clinical details and outcome are shown in table 1. No immediate complications occurred as a result of the invasive procedures. Results of laboratory studies on fetal blood are shown in table 2. The karyotype in all in fetal blood was normal, and a normal karyotype was obtained from triplet fetuses B and C by culture of amniotic fluid cells obtained by amniocentesis. Each pregnancy was concordant for fetal sex. Clotting studies (platelets, thrombin time, fibrinogen, and fibrin degradation products) from the surviving fetus of case 2 were normal.

Six and 18 ml of donor blood (packed cell volumes of 0·72 and 0·87) were transfused into the donor twins of cases 4 and 5, respectively. Kleihauer testing of fetal blood then obtained from the recipient twins 25 minutes (case 4) and 16 minutes (case 5) later showed that 145 and 70 cells/1000, respectively, had stained as adult cells. In both cases the needle tip was seen clearly within the umbilical vein of the recipient suggesting that appreciable maternal contamination was unlikely. This procedure was carried out in case 4 to document the sharing of circulations in utero, in view of the similar fetal haemoglobin concentrations. In contrast, this was done in case 5 at 36 weeks to improve the condition of the fetus before delivery by correcting polycythaemia and anaemia. In case 5 the recipient was sampled first, polycythaemia was confirmed, and 30 ml of blood was with-

Table 1 Clinical details and outcome in six pregnancies complicated by fetofetal transfusion syndrome. Gestational age at time of blood sampling shown in parentheses

	Case 1 (23 weeks)		*Case 2 (27 weeks)*		*Case 3 (21 weeks)*		*Case 4 (21 weeks)*		*Case 5 (36 weeks)*		*Case 6 (26 weeks)*		
	Fetus A	*Fetus B*	*Fetus A*	*Fetus B*	*Fetus A*	*Fetus B*	*Fetus A*	*Fetus B*	*Fetus A*	*Fetus B*	*Fetus A*	*Fetus B*	*Fetus C*
Volume of amniotic fluid	Increased	Nil	Increased	Normal	Increased	Nil	Increased	Nil	Increased	Reduced	Increased	Normal	Reduced
Ultrasonic growth (biparietal diameter and abdominal circumference)	Normal	≤5th Centile	Intrauterine death	≤5th Centile	Normal	<5th Centile	Normal	<5th Centile	Normal	<5th Centile	Normal	Normal	<5th Centile
End diastolic frequencies detected by umbilical artery Doppler	Not measured	Not measured	Intrauterine death	Not measured	Present	Present	Present	Absent	Present	Present	Absent	Present	Absent
Hydrops present	No	No	Yes	Yes	No	No	No	No	Yes	No	Yes	No	No
Outcome	Miscarried one day later		Preterm labour: Stillbirth	Neonatal death	Intrauterine deaths six days later		— Miscarried four days later	Intrauterine death three days later	Live birth Elective caesarean section next day	Live birth	Preterm labour five days later Stillbirth	Neonatal death day 8	Stillbirth
Birth weight (g)	560	420	(444)	730	355	200	480	400	2510	1658	1680	878	430
Extramedullary haemopoiesis at necropsy	No	No	No	Yes	No necropsy		Yes	No	Not applicable		Yes	No	Yes
Heart: body weight ratio at necropsy	0·015	0·005	(0·013)	0·007	No necropsy		0·012	0·003	Not applicable		0·006	0·007	0·005

Table 2 Laboratory results on fetal blood. Case 2—fetus A, Case 3—fetus B, and Case 6—fetuses B and C did not have samples taken. Results above the normal range are shown in bold type, those below in italics

	Case 1		*Case 2*	*Case 3*	*Case 4*		*Case 5*		*Case 6*
	Fetus A	*Fetus B*	*Fetus B*	*Fetus A*	*Fetus A*	*Fetus B*	*Fetus A*	*Fetus B*	*Fetus A*
Haematology:									
Packed cell volume	**0·53**	0·47	0·36	**0·52**	0·45	**0·47**	**0·68**	*0·31*	**0·61**
Haemoglobin (g/l)	**186**	**166**	128	**180**	110	**153**	**230**	*99*	**185**
Nucleated red cell count ($\times 10^9$/l)	**52·9**	**42·0**	7·3	8·7	**1·9**	**4·8**	**2·9**	**17·0**	27·5
Reticulocytes ($\times 10^9$/l)	**0·71**	**0·51**	**0·45**	**0·48**	0·37	**0·67**	**0·46**	**0·31**	**0·37**
As %	**16·5**	**13·5**	**19·0**	**13·5**	**11·0**	**19·7**	**7·8**	**12·2**	**8·6**
Blood gases:									
pH	*7·27*	*7·30*	7·38	7·43	7·37	*7·23*	7·34	7·39	*7·19*
PCO_2 (kPa)	**6·66**	**5·98**	5·11	4·68	**5·96**	**8·81**	5·16	4·93	**7·15**
PO_2 (kPa)	*1·86*	*2·39*	5·10	*3·63*	*3·02*	*1·52*	4·47	4·08	*1·41*
Base excess	−4·5	−4·3	−1·8	0	−0·7	−2·0	−5·2	−4·3	*−8·4*

Fetus A, Case 4, had samples taken after transfusion of the sibling fetus.

drawn to reduce the fetal packed cell volume. The donor was then sampled and transfused, its packed cell volume increasing from 0·31 to 0·36. The volume of red cells transfused was calculated as less than that removed from the recipient in case immediate fetofetal transfusion worsened the recipient's polycythaemia. The recipient was then resampled, a further 6 ml of blood withdrawn, and 15 ml normal saline instilled intravascularly. Dilutional exchange transfusion in utero was abandoned at this stage because of a transient bradycardia. Umbilical artery flow velocity waveforms remained normal throughout. At elective delivery the next day, Kleihauer testing of the recipient's cord blood showed that 160/1000 cells were adult. The donor's neonatal course was uncomplicated, but the recipient required dilutional exchange transfusion after the packed cell volume peaked at 0·72; the infant subsequently developed necrotising enterocolitis, and recovered with conservative management.

There were 11 fetal or perinatal losses, giving a total perinatally related wastage of 85%.

The diagnostic criteria by which all these pregnancies were considered to have been complicated by fetofetal transfusion syndrome are shown in table 3. Discordant growth and amniotic fluid volumes in the presence of monochorionic placentas was seen on ultrasonography in all, and hydrops was noted in three recipient fetuses. After delivery, fetofetal transfusion syndrome was confirmed in each by differences in fetal weight of 20% or more. In the four that underwent necropsy, extramedullary haemopoiesis in the donor, with or without relative macrocardia in the recipient, provided additional confirmation of the diagnosis.[26] In the remaining two, additional confirmation was achieved by the difference in haemoglobin concentrations (case 5), and the clinical appearance at delivery (case 3).[3]

Discussion

Fetal haematology in these pregnancies with fetofetal transfusion syndrome suggests that, unlike postnatally, a haemoglobin difference of 50 g/l or more is not necessarily found in utero. In this series, only one of three twin pregnancies in which both fetuses were sampled showed major differences in haemoglobin values (case 5), and this was in a pregnancy with a chronic well compensated fetofetal transfusion syndrome sampled late in the third trimester. In contrast, the remaining two without major differences in haemoglobin concentrations were at much earlier gestations (21 and 23 weeks). Case 1 showed a degree of polycythaemia in both fetuses, while in case 4, polycythaemia was found in the donor and not in the recipient. In this atypical case, however, a marginal velamentous cord insertion with abnormal umbilical Doppler flow implicated deficient placentation in the donor's intrauterine growth retardation, itself a known cause for polycythaemia. In the two multiple pregnancies in which only the recipient was sampled, raised haemoglobin concentrations and packed cell volumes were consistent with the diagnosis of fetofetal transfusion syndrome. Because of the above discrepancies, and the existence of other mechanisms for polycythaemia, however, isolated polycythaemia in one twin or triplet cannot be considered diagnostic.

Rausen *et al* were the first to suggest that a

Table 3 Presence of diagnostic features in six pregnancies complicated by fetofetal transfusion syndrome

Case No	*Ultrasound measurements of biparietal diameter[39] and abdominal circumference[40] ≤5th centile in one fetus and not the other(s) (discordant growth)*	*Polyhydramnios in one sac and not the other(s) (discordant volume)*	*Hydrops in recipient*	*Difference in haemoglobin concentration of ≥50 g/l between fetuses*	*Signs of shared circulation**		*Difference in birth weight of ≥20% between fetuses*	*Extramedullary haemopoiesis in donor*	*Difference in heart:body weight ratio of ≥50% between fetuses at necropsy*
					In vivo	*In vitro*			
1	Yes	Yes	No	No	Not applicable	Yes	Yes	No	Yes
2	Yes	Yes	Yes	Not applicable	Not applicable	No	Not applicable†	Yes	Yes
3	Yes	Yes	No	Not applicable	Not applicable	Not applicable	Yes	Not applicable	Not applicable
4	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes
5	Yes	Yes	Yes	Yes	Yes	No	Yes	Not applicable	Not applicable
6	Yes	Yes	Yes	Not applicable	Not applicable	No	Yes	Yes	No

*Signs of shared circulation in vivo means that adult red cells were detected in the recipient after adult blood had been transfused into the donor, whereas in vitro shared circulation refers to the presence of placental superficial anastomoses.

†Retrospective calculation using centile charts of the estimated fetal weight of the surviving twin suggested a difference of ≥20% at that time.

difference of 50 g/l in neonatal haemoglobin concentrations was diagnostic of fetofetal transfusion syndrome.[4] In their series, 19 of 130 monochorionic twins had such a difference, while no set of dichorionic twins had a discrepancy of greater than 33 g/l. This criterion for the diagnosis of fetofetal transfusion syndrome has since been widely adopted.[5–7] Haemoglobin discordance has, however, also been reported in dichorionic twins.[5] A recent study reported that seven of 13 sets of twins with neonatal haemoglobin discordance of more than 50 g/l were in fact dichorionic.[27] Fetofetal transfusion syndrome occurs almost exclusively in monochorionic placentas, although rarely vascular communications have been reported in fused dichorionic placentas.[2] On the other hand, intrauterine growth retardation is common in dichorionic twins, so that haemoglobin discordance in dichorionic twins is far more likely to reflect polycythaemia secondary to intrauterine growth retardation. Tan *et al* described 35 sets of twins with the required haemoglobin difference, but made no mention of placental chorionicity.[6] In nine of their pregnancies the polycythaemic twin was smaller than the anaemic twin, suggesting that several cases of dichorionic twins with intrauterine growth retardation were falsely diagnosed as having fetofetal transfusion syndrome. Although the standard neonatal criteria have recently been questioned,[27] it seems reasonable to apply them at birth provided that they are applied only to monochorionic twins in which the heavier twin has the higher haemoglobin concentration.

This discrepancy between the classic postnatal findings and our observations made in utero may be related to differences in gestation or the duration and degree of shunting, or both. Presentation in the midtrimester suggests a more acute form of fetofetal transfusion syndrome in which there may have been insufficient time for haematological compensation. Alternatively, the considerable mixing of the circulations implicit in large shunts may not necessarily be reflected in differences in haemoglobin concentrations.

Evidence of erythroblastosis (increased nucleated red cells and reticulocytes) was found in all the fetuses sampled, usually to a greater extent in the donor. Erythroblastaemia in the recipient could result either from fetofetal transfusion of donor erythroblasts,[28] or from the action of donor erythropoietin on the recipient marrow.[29] This latter suggestion would explain our finding of extramedullary haemopoiesis in some recipients. In addition, erythroblastaemia in the recipient may be secondary to hypoxia, which we found in four of five of the recipients sampled.

An alternative approach to diagnosis is in vivo demonstration of appreciable sharing of circulations. This was achieved in two pregnancies by giving a readily detectable safe marker with subsequent demonstration of its passage in substantial quantities into the recipient. The finding of a considerable proportion of adult red cells in the recipients (14·5 and 7% of red cells in cases 4 and 5, respectively) after only short intervals suggests that a large amount of transfer occurred rapidly. We found no such transfer in an Rh alloimmunised monochorionic twin pregnancy uncomplicated by fetofetal transfusion syndrome in whom intrauterine transfusion was indicated for fetal anaemia, but this is anecdotal evidence and the technique warrants further evaluation in the diagnosis of fetofetal transfusion syndrome in utero.

Fetal blood sampling in this series supplied additional information about fetal wellbeing. Four of nine fetuses were acidaemic, while two others in addition to these four were also hypoxaemic. As with other high risk pregnancies, fetal acid base balance may contribute to the assessment of fetal compromise and to the timing of intervention or delivery, or both.

This series confirms the extremely high mortality (85%) in fetofetal transfusion syndrome,[13 30] and reflects both the severity of disease and the early gestation of the patients referred. All fetal losses in this series occurred before 28 weeks, and survival after diagnosis of fetofetal transfusion syndrome in the second trimester is most unusual. A 100% perinatal mortality rate has been reported in a series of eight monochorial pregnancies complicated by polyhydramnios in the midtrimester.[30] Thus our finding of fetal hypoxaemia or acidaemia, or both, in four of five pregnancies that ended in fetal loss is hardly surprising. It seems unlikely that the losses in our series were related to the invasive procedures; most complications of fetal blood sampling occur immediately, and none were observed here.

In view of the uniformly poor outcome of fetofetal transfusion syndrome in the second trimester, aggressive therapeutic measures may be warranted. Laser ablation of superficial placental anastomoses has been investigated in animal studies[31]; it is, however, the deeper and not the more accessible superficial anastomoses that seem to be responsible for the haemodynamic imbalance in fetofetal transfusion syndrome.[9 28] Selective fetocide of one twin has been reported in three pregnancies with fetofetal transfusion syndrome, two of which resulted in live singletons born at full term.[32–34] In no case was there evidence of hydrops or other fetal compromise, this drastic procedure being carried out primarily to allow prolongation of pregnancies threatened by gross polyhydramnios. In such cases simpler treatments deserve consideration. Serial amniocentesis has been recommended to allow symptomatic relief and to delay preterm labour,[35] although some have found it unsuccessful.[3 33] We have recently shown that removal of small quantities of amniotic fluid in gross polyhydramnios restores raised amniotic fluid pressure to normal.[25] Although this procedure seems to alleviate maternal symptoms, it is not known how long thereafter it takes for amniotic pressure to rise again. Treating the mother with indomethacin has also been suggested to alleviate polyhydramnios,[36] as it reduces fetal urine output.[37] This approach is contraindicated in the presence of oligohydramnios in one twin,[36] however, a feature in five of the six pregnancies reported here.

An alternative to selective fetocide is suggested by the report of Vetter and Schneider[38] who noted resolution of fetofetal transfusion syndrome in two pregnancies after iatrogenic puncture of a chorionic plate vessel at amniocentesis with consequent intra-amniotic haemorrhage. Repeated phlebotomy of the recipient and transfusion of the donor has been considered as a treatment for fetofetal transfusion syndrome.[33] The degree of shunting that we found in vivo in cases 4 and 5 was substantial, however, indicating that serial 'give and take' transfusion would be unlikely to be of long term benefit. Given that the hydropic recipient twin seems relatively more at risk than its donor, venepuncture of the recipient alone warrants consideration as a treatment for fetofetal transfusion syndrome.

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Circulatory effects of fast ventilator rates in preterm infants

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Abstract

High frequency positive pressure ventilation has been suggested to result in a lower incidence of respiratory complications in preterm infants with idiopathic respiratory distress syndrome compared with ventilation at conventional rates. A possible disadvantage is compromise of the infant's cardiovascular condition secondary to inadvertent positive end expiratory pressure (PEEP). In a group of 20 such infants treated with high frequency positive pressure ventilation (rates of up to 100/minute) and paralysed, changes in arterial blood pressure and cerebral blood flow velocity were largely influenced by changes in arterial blood gases, and no effect could be attributed to inadvertent PEEP. In addition, the observed fall in both arterial carbon dioxide and oxygen tensions could be readily predicted for theoretical reasons. Under certain conditions at the fastest rates used, cerebral blood flow velocity was significantly influenced by changes in blood pressure, which may indicate impaired cerebrovascular regulation. Though other factors (such as the severity of the infants' illness or the use of paralysis) may have been responsible for this apparent blood pressure passivity, the role of high frequency positive pressure ventilation in such infants warrants further study.

During recent years the type of infant treated in neonatal units has changed,[1] and the extremely immature infants now encountered regularly are at great risk from the respiratory complications of idiopathic respiratory distress syndrome. In addition, neonatal ventilators are now more sophisticated and these factors have led to different patterns of ventilation being tried in attempts to minimise the complications of respiratory support.

The use of one such alternative, high frequency positive pressure ventilation, has been increasing in neonates, because of a reported reduction of harmful interaction with the infant's own respiratory efforts,[2] as well as an improvement in oxygenation in unparalysed infants.[3] Concerns about high frequency positive pressure ventilation have centred around the production of inadvertant positive end expiratory pressure (PEEP),[4] resulting from insufficient time for the lungs to empty during the expiratory phase. This may lead to harmful secondary effects on both the lungs and the cardiovascular system. The former effect may impair blood gases and the latter may produce a number of secondary problems, for example decreased venous return,[5] or restriction of left ventricular filling.[6]

Several factors are likely to influence the occurrence of inadvertant PEEP and whether its effects are transmitted to the cardiovascular system. Theoretically, it is more likely to occur in paralysed infants who cannot expire actively, and also if the degree of air leak around the endotracheal tube is relatively small. Mechanical transmission of inadvertant PEEP to the cardiovascular system will be affected by lung compliance,[7] which will vary with the stage of idiopathic respiratory distress syndrome. To assess the magnitude of these potential cardiovascular effects we have studied the changes produced by high frequency positive pressure ventilation on arterial blood pressure and cerebral blood flow velocity in a group of paralysed infants in the acute phase of idiopathic respiratory distress syndrome to see whether any of the changes that occurred could be attributed to inadvertent PEEP.

Patients and methods

The study was carried out on the neonatal unit at the Leicester Royal Infirmary between June 1988 and August 1989. Twenty infants were studied on one occasion each. Their birth weights ranged from 720–2420 g (median 1480 g) and their gestational ages from 25–34 weeks (median 30 weeks). The median age at the time of study was 54·5 hours. Median peak inspiratory pressure at the time of the study was 24 cm H_2O and median inspired oxygen concentration was 0·73. Full details of the infants are given in the table.

Infants were considered eligible for the study if they were being ventilated for respiratory distress syndrome and were paralysed with pancuronium to facilitate ventilation. The decision to use pancuronium was based on the inability to ventilate the infant satisfactorily at either slow or fast rates and was made independently by the clinicians responsible for the infants' care. All infants had indwelling arterial catheters (either umbilical or radial) for clinical reasons and were intubated orally with shouldered endotracheal tubes. An infant was excluded if a pneuomothorax was present, if there was pre-existing intraventricular haemorrhage, or if the infant's condition was unstable.

The study was approved by the Leicestershire district ethics committee. Informed parental consent was obtained before all studies and parents were encouraged to be present for the duration of the study. The following variables were recorded:

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Details of infants at time of study

Case No	*Gestation (weeks)*	*Birth weight (g)*	*Age at study (hours)*	*Fractional inspiratory oxygen*	*Peak inspiratory pressure (cm H_2O)*	*Positive end expiratory pressure (cm H_2O)*
1	29	900	124	0·86	22	2
2	30	1500	77	0·75	28	2
3	32	2000	66	0·60	20	2
4	32	2070	66	0·80	23	3
5	31	1990	73	0·70	26	4
6	30	1700	14	1·00	32	4
7	30	1280	81	0·98	31	3
8	29	1360	43	0·78	25	2
9	32	2070	20	0·58	20	2
10	26	880	57	0·96	26	2
11	32	1790	21	0·84	35	4
12	30	1550	49	0·70	25	2
13	27	1100	216	0·35	15	2
14	29	1400	22	0·58	20	3
15	25	750	16	1·00	20	2
16	31	1450	38	0·58	20	4
17	27	1060	144	0·65	20	3
18	30	720	86	0·50	20	3
19	31	1600	23	0·46	28	2
20	34	2420	52	1·00	32	3

Cases 6, 10, and 15 died of idiopathic respiratory distress syndrome.

CEREBRAL BLOOD FLOW VELOCITY AND ARTERIAL BLOOD PRESSURE

In three of the infants cerebral blood flow velocity recordings were made from the anterior cerebral artery using an ATL 600 duplex Doppler system with a 724A probe (pulse echo 7·5 MHz, Doppler frequency 5 MHz). One anterior cerebral artery was visualised in the sagittal plane through the anterior fontanelle and signals were recorded from a point midway between the most inferior border of the corpus callosum and the vessel's origin in the circle of Willis. Care was taken to ensure that the angle of insonation of the vessel was less than 10°.

For these recordings the Doppler sample value was set to 3 mm and the high pass wall filter at its lowest value (100 Hz). Doppler signals were recorded on to digital audiotape and subsequently replayed through a fast Fourier transform analyser.[8] The maximum frequency envelope of 20 or more consecutive beats was extracted and calibrated in terms of velocity using the Doppler equation. The mean velocity over the cardiac cycle was estimated by halving the mean value of the maximum velocity over the cycle.[9] In these infants, a permanent record of blood pressure was made on a chart recorder.

The remaining 17 infants studied had cerebral blood flow velocity measurements recorded from one middle cerebral artery using a miniature probe attached to the infant's skin, which we have previously described.[10 11] This system also collects data about blood pressure from the indwelling arterial line and displays the information graphically at the cotside. This obviates the need to handle the infant repeatedly to make estimations of cerebral blood flow velocity. Results may also be retrieved off line at a later stage. In all infants, mean arterial blood pressure was recorded at each stage of the study.

RESPIRATORY RECORDINGS

Ventilator pressures were recorded from the endotracheal tube through a 19 gauge needle connected to a pressure transducer. Tidal volumes were recorded using a Gaeltec pneumotachograph (with a bias flow system to eliminate dead space) combined with a differential pressure transducer and integrator as described previously.[12] All signals were recorded on a chart recorder for later analysis.

STUDY PROCEDURE

After the recording apparatus had been attached, a period of 15 minutes was allowed for stabilisation of the infant's condition. All infants were then studied at three ventilatory rates: 30–40, 50–60, and 100/minute, using a Sechrist ventilator (model IV-100B). Peak inspiratory pressure, PEEP, and inspiratory: expiratory ratio were kept constant. The rate change sequence was initially an increase in all cases, as all infants entered the study while being ventilated at either 30–40 or 50–60/minute. Infants entering at 30–40/minute had their rate increased by at least 20/minute as a first step. Those entering at 50–60/minute were increased to 100/minute and then reduced to 30/minute as a last stage before returning to their baseline rate.

At 100/minute, a second set of recordings were made with the pneumotachograph bias flow switched off. This manoeuvre was performed to enable comparison of the cardiovascular effects of the rate change at different arterial carbon dioxide pressure ($PaCO_2$). A 12 minute stabilisation period was allowed after each rate change before measurements were made of mean arterial blood pressure, cerebral blood flow velocity, tidal volume, peak inspiratory pressure, and PEEP. Even at the fastest rates, the ventilator pressure wave flattened out. At each stage 0·1 ml of blood was taken through the arterial line for measurement of $PaCO_2$ and arterial oxygen pressure (PaO_2).

STATISTICAL ANALYSIS

Parametric statistical tests were used. Paired *t* tests were used to compare observed values of PaO_2, $PaCO_2$, mean arterial blood pressure, and cerebral blood flow velocity at the four ventilator settings. No adjustment was made for multiple comparisons. The principal findings would not have been altered by this adjustment, which was judged unnecessary because of the small number of comparisons being made. Linear regression analysis was used to examine the factors influencing changes in cerebral blood flow velocity. A step down analysis was used, incorporating $PaCO_2$, PaO_2, and mean arterial blood pressure as potential explanatory variables. The adequacy of the linear model was checked by plotting residuals.

Results

PaO_2 changed little at the different ventilator settings, but was significantly lower at 100/minute than at 30/minute (p=0·043) (fig 1). $PaCO_2$ was significantly lower at 60/minute (p=0·0003), and at 100/minute (p=0·016), than at 30/minute (fig 2). With the bias flow turned off at 100/minute, however, $PaCO_2$ was not significantly different from that observed at 30/minute (p=0·7). There was a substantially higher $PaCO_2$ at 100/minute with the bias flow turned off than at the same rate with the pump on (p=0·004).

There was a slight downward trend in mean arterial blood pressure at increasing ventilation rates, which was significant at 100/minute compared with 30/minute (p=0·043). Turning the bias flow off at 100/minute, however, resulted in a clear increase in mean arterial blood pressure (p=0·003) compared with the same rate with the pump on (fig 3). The statistical analysis suggested that this did not depend significantly on the accompanying changes in blood gases.

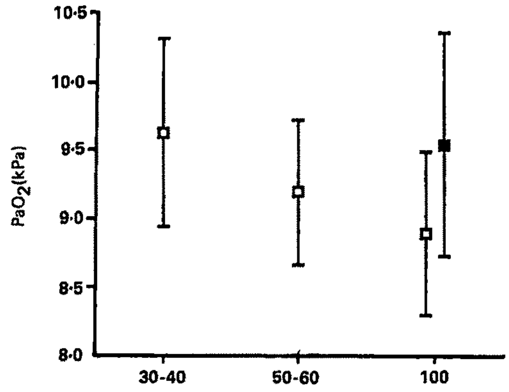

Figure 1 Mean (SEM) PaO_2 at different ventilator rates. Open symbols represent bias flow on, shaded symbol represents bias flow off.

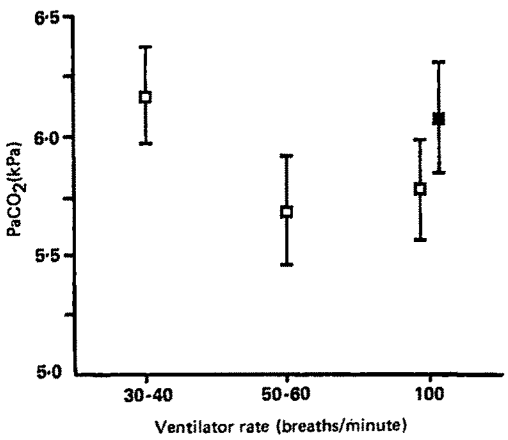

Figure 2 Mean (SEM) $PaCO_2$ at different ventilator rates. Open symbols represent bias flow on, shaded symbol represents bias flow off. Increasing minute ventilation resulted in an initial fall in $PaCO_2$, until tidal ventilation became insufficient (compare with figure 5).

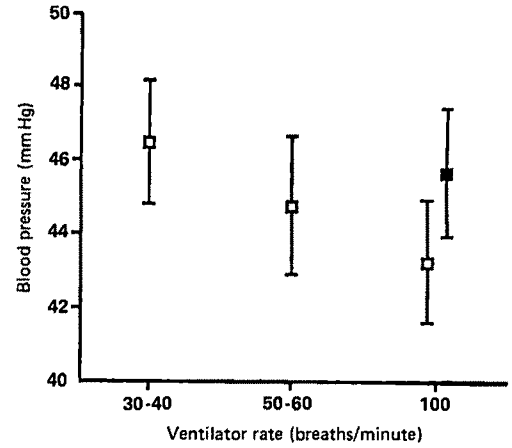

Figure 3 Mean (SEM) blood pressure at different ventilator rates. Open symbols represent bias flow on, shaded symbol represents bias flow off.

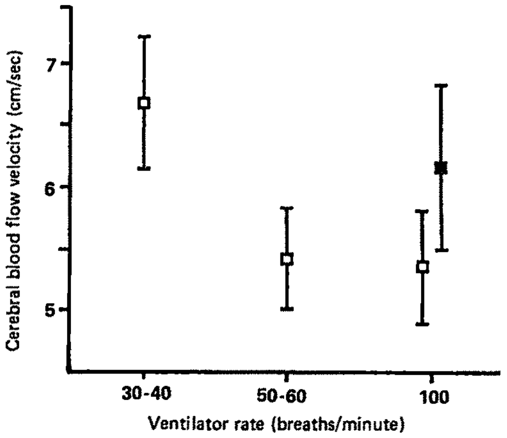

Figure 4 Mean (SEM) cerebral blood flow velocity at different ventilator rates. Open symbols represent bias flow on, shaded symbol represents bias flow off.

Cerebral blood flow velocity fell as ventilator rate was increased from 30 to 60/minute (p=0·0017), but showed no further fall on further increasing the rate to 100/minute (p=0·74). Removal of the bias flow at this rate resulted in a clear rise in cerebral blood flow velocity (p=0·008) to the level observed at 30 breaths/minute (fig 4). Stepwise linear regression indicated that two separate factors were associated with the observed changes in cerebral blood flow velocity. Firstly, as ventilator rate was changed from 30 to 100/minute, the change in cerebral blood flow velocity was closely associated with the change in $PaCO_2$ (p=0·008), but was independent of changes in PaO_2 or blood pressure. The variation in $PaCO_2$ alone was sufficient to account for 30% of the variability in the change of cerebral blood flow velocity.

Secondly, the rise in cerebral blood flow velocity on removal of the bias flow at 100/minute was not related to the change in $PaCO_2$, but was significantly dependent on the change in mean arterial blood pressure ($p=0{\cdot}014$). Once the change in mean arterial blood pressure had been included in the regression model, the effect of the change in $PaCO_2$ was not significant ($p=0{\cdot}14$); neither was the effect of the change in PaO_2 ($p=0{\cdot}9$). Twenty nine per cent of the variability in the change in cerebral blood flow velocity resulting from the removal of the bias flow was attributable to the associated change in mean arterial blood pressure.

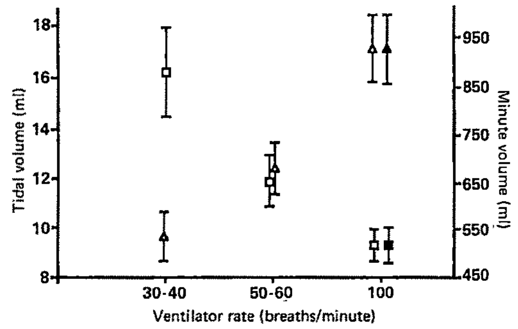

Figure 5 Mean (SEM) tidal (□) and minute (△) volumes with increasing ventilator rates. Open symbols represent bias flow on, shaded symbols represent bias flow off.

Discussion

There is relatively little data on the effects of inadvertent PEEP in preterm infants which occurs even at relatively slow ventilator rates.[4] Our findings indicate that the use of high frequency positive pressure ventilation in paralysed infants with stable idiopathic respiratory distress syndrome does not result in adverse haemodynamic effects or changes in blood gases that might be attributable to inadvertent PEEP.

In a study such as this, standardisation of patients by stage of disease is clearly important. The criteria for the use of pancuronium were well defined and all the infants were studied when their ventilatory requirements were stable.

Important factors determining the production of inadvertent PEEP are lung compliance and the amount of air leak around the endotracheal tube.[4] We attempted to measure static compliance in all of our infants using a prolonged inspiration technique, but were unable to prevent significant air leaks in 18 of the infants studied, despite the use of shouldered endotracheal tubes. The two infants in whom successful measurements were made had compliances of 0·5 and 0·6 ml/cm H_2O, respectively. As major leaks around the endotracheal tubes occurred in most of our infants, it seems likely that this effect limits the tendency to develop inadvertent PEEP. As our clinical practise in regard to type and size of endotracheal tube used is similar to that in many neonatal units, we believe that inadvertent PEEP does not pose a clinically important problem in paralysed infants.

A potential criticism of our method in view of the air leak is that rather than measuring tidal volumes, we were in fact merely recording changes in air leak. Though this may be true to some extent, the rise in minute volumes we recorded (fig 5) were accompanied by concurrent falls in $PaCO_2$ between 30 and 60 breaths/minute, which would not have occurred if the changes measured had merely represented alterations in air leak. The subsequent rises in $PaCO_2$ between 60 and 100 breaths/minute despite further rises in minute volume probably resulted from the fall in tidal volume at the higher rate (fig 5) rendering it less effective.

The significant fall in PaO_2 between slow and fast rates may also be predicted from theoretical principles, as increasing ventilator rate alone (that is, producing an increased number of ventilator up strokes/unit time) will result in a fall in mean airway pressure, thus directly affecting oxygenation.

Our findings are in contrast to those of Greenough *et al*, who found that the use of high frequency positive pressure ventilation did not result in significant changes in arterial blood gases.[13] We suspect that these findings result from differences in methods. Firstly, half the infants in this study were ventilated with Bourns ventilators, which in our experience are less efficient at rates of 100/minute. Secondly, flow rates were altered to maintain mean airway pressure while keeping inspiratory:expiratory ratios and peak inspiratory pressure constant. To do this, an increase in PEEP must have been required (not stated in the paper), which would have negated the changes in $PaCO_2$ and PaO_2 that we observed.

It has been recognised in animal studies since the last century that changes in blood gases have an important influence on the cerebral vasculature,[14] but the full extent of this effect on cerebral blood flow had to await reliable methods of estimating cerebral blood flow in humans.[15] Models for cerebral injury in preterm neonates have hypothesised that there is impaired cerebrovascular regulation,[16] with vascular 'watershed' regions notably in the periventricular white matter most at risk from ischaemia. If the normal regulatory mechanisms fail to operate, 'pressure passive' blood flow may result, but evidence for this is scanty and has been obtained from heterogeneous groups of infants.[17]

The finding that in particular circumstances changes in mean arterial blood pressure seemed to exert more influence on cerebral blood flow velocity (and thus actual cerebral blood flow)[18] than $PaCO_2$ was unexpected. Though care must be taken in attaching clinical importance to significant results generated unexpectedly from statistical analysis, several points are of note. Firstly, for this apparent relationship to have resulted from the statistical analysis alone is unlikely, as not only was the significance of the effect of mean arterial blood pressure gained, but that of $PaCO_2$ lost. Secondly, an effect secondary to the order of rate changes during the study would have been expected to have an opposite effect to that seen. Thirdly, this effect was observed at carbon dioxide tensions encountered at other times in the study and it is therefore unlikely that maximal cerebrovascular dilatation secondary to changes in $PaCO_2$ had been achieved. Were this the case, changes in blood pressure may indeed have had a more prominent influence on cerebral blood flow, resulting in pressure passive cerebral blood flow.

Finally, this group of infants represents some of the illest treated in our unit, and the apparent dependence of cerebral blood flow velocity on mean arterial blood pressure may actually represent impaired cerebrovascular regulation. It is, however, important to bear in mind that $PaCO_2$ acts both by direct and indirect (that is, sympathetically mediated) mechanisms on the cardiovascular system,[19] and thus may still be the governing factor in the responses observed. In addition, we may have been observing the effect of other factors—for example, those secondary to the use of paralysing agents.

The rise in PaO_2 observed with the bias flow turned off was also unexpected. We suspect that this too may have resulted from an indirect effect of the change in $PaCO_2$ mediated by changes in mean arterial blood pressure. This would in turn raise left atrial pressure and alter shunting. Interestingly, none of the surviving infants subsequently developed ultrasound evidence typical of preterm cerebral injury.[20]

In summary, the use of high frequency positive pressure ventilation in paralysed infants does not seem to result in adverse cardiovascular effects that are attributable to inadvertent PEEP. In addition, it results in statistically significant (though not necessarily clinically relevant) changes in arterial blood gases, which would appear to be readily predictable from theoretical considerations. Under certain conditions these infants had changes in cerebral blood flow velocity that may have been to a certain degree dependent on changes in blood pressure. The use of ventilator rates greater than 50 or 60 breaths/minute in paralysed infants seems to confer no advantage and may in fact be detrimental. Clearly their role in such infants requires further study.

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Follow up of premature babies treated with artificial surfactant (ALEC)

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Abstract

Of 235 survivors who had taken part in a randomised trial of artificial surfactant and who were born in Cambridge, follow up information was available for 231 (98%) infants. In 12 cases information came from local doctors; all others were assessed at 9 and 18 months (n=212) or 9 months only (n=7). There was no difference between those who had been treated with surfactant and control babies in the incidence of neurological impairment, mental impairment, respiratory infections, allergies, or hospital admissions up to 18 months after full term. In those born before 30 weeks' gestation (where surfactant most improves survival) the number of surviving randomised children who were normal was 35 of 61 in the treated group (57%) compared with 25 of 61 in the control group (41%). Improved neonatal survival after prophylactic surfactant treatment is not associated with an increased incidence of neurodevelopmental impairment.

Artificial surfactant (artificial lung expanding compound, Pumactant, Britannia Pharmaceuticals) composed of dipalmitoylphosphatidylcholine and unsaturated phosphatidylglycerol in a ratio of 7:3 has been used successfully in one case controlled study[1 2] and two randomised control trials.[3 4] In the last of these, a multicentre trial, a reduction in neonatal mortality for babies between 25 and 29 weeks' gestation, from 27% to 14% was shown. There was also a reduction of one third in the incidence of parenchymal brain haemorrhages from 24% to 16%, and a significant reduction in the need for more than 30% oxygen and the amount of artificial ventilation required. There has been concern that improved survival of these very premature babies may result in an increased number of impaired children. This trial presents the follow up of the survivors treated in Cambridge from the first randomised trial.[3]

Patients and methods

All surviving children from the trial were invited for follow up examinations by RM 9 and 18 months after their expected date of delivery. The examiner did not know any details about the infants' neonatal progress, nor whether they had been randomised to receive treatment with surfactant or to be controls. At 9 months a medical history and full physical examination were undertaken, including the neurological examination described by Amiel-Tisson and Grenier.[5] The developmental screening inventory described by Knobloch *et al* was also used.[6] The score for each area of development was derived from the child's age corrected for prematurity (that is, chronological age minus the time from delivery to expected date of delivery). An overall development quotient was calculated as the mean of the quotients for the five individual areas (adaptive, motor, fine motor, language, and personal/social).

At 18 months a history was taken and the child had another physical examination. The Bayley mental and motor scales were administered and the mental development index and psychomotor development index calculated.[7] The mental scale, like most tests of intellectual development in small children, relies heavily on fine motor skills appropriate for age. In children with cerebral palsy, therefore, the results often bear little relation to intellectual prowess. The academic scale of Developmental Profile II[8] for which an intelligence quotient (IQ) equivalent can be calculated relies little on fine motor skills at this age, so it was used for all infants in addition to the Bayley mental scale. Scores for all these tests were also calculated for the age corrected for prematurity. Mental impairment was diagnosed when the child's score was more than two standard deviations below the population mean. Social class of the family was coded using the Registrar General's classification, social class III being subdivided into nonmanual and manual.[9] The child's rank was recorded as his birth order in the living children in the family, with twins assigned equal rank.

Student's t test, the χ^2 test, and linear regression were used for statistical analysis of the results.

Results

Of 276 eligible infants included in the surfactant trial, 244 (119 controls and 125 treated with surfactant) survived to leave the neonatal unit. Nine of these infants died later, five controls (two of chronic lung disease, one of sudden infant death sydrome (SIDS), one of Wilms' tumour, and one of toxic shock syndrome), and four treated with surfactant (three of chronic lung disease, and one of SIDS). There were 235 babies eligible for follow up. Four were lost to follow up: all were children whose parents had been posted overseas. They were all girls and included one control and three who had been treated with surfactant; none were born at less than 30 weeks' gestation. It was not known whether they survived. In total, 231 infants were known to survive to 18 months after full

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term: 114 controls (54 boys) and 117 treated with surfactant (58 boys). Of these, 212 (92%) were seen at 18 months: 107 controls and 105 treated with surfactant; a further seven were only seen at 9 months, before they moved from the area; thus 219 (95%) were seen and examined by RM. The remaining 12 children (six in each group) had left the area, but information on their developmental and neurological prowess was obtained from their local doctors.

CHILDREN BORN AT LESS THAN 30 WEEKS' GESTATION

Of the 122 infants born at less than 30 weeks' gestation who entered the trial (61 in each group) there were 85 survivors who were all seen or reported on at 18 months (47 treated and 38 controls).

OUTCOME OF CHILDREN NOT SEEN AT FOLLOW UP

Ten of the 12 children who were not seen by RM were reported to be developmentally normal (five in each group): of the other two, one (a control) was reported to have mild spastic diplegia but not mental impairment and the other (a treated baby) was microcephalic and mentally impaired.

OUTCOME OF CHILDREN SEEN ONLY AT 9 MONTHS

Of the seven infants seen only at 9 months, six had developmental quotients well within the normal range; they were reported as developmentally normal at 18 months. One, a control infant, had Prader-Willi syndrome and was mentally impaired; he was excluded from analysis of mental impairment as his impairment was the result of a recognised congenital abnormality.

NEUROLOGICAL OUTCOME

Twenty three children were diagnosed as having neurological impairment: nine of 114 were controls (8%) and 14 of 117 had been treated with surfactant (12%).

Among the infants of less than 30 weeks' gestation, neurological impairment was diagnosed in six of 38 controls (16%) and nine of 47 treated infants (19%), and in those of 30 weeks' gestation or more the incidence was three of 76 among the controls (4%) and five of 70 in the treated infants (7%). There was no significant difference between treated and control children.

Severity of impairment was assessed functionally. Thus children not able to sit unsupported at 18 months were classified as 'severely impaired', those not able to pull up to standing or walk with hands held as 'moderately impaired', and those able to do all these things but having abnormal neurological signs as 'mildly impaired'. In the control group impairments were: severe (n=4), moderate (n=1), mild (n=4), compared with severe (n=7), moderate (n=2), and mild (n=5) in the treated group. In the babies born before 30 weeks' gestation the impairments were: severe (n=3), moderate (n=1), mild (n=2) among the controls, and severe (n=5), moderate (n=1), mild (n=3) in those who were treated.

DEVELOPMENTAL OUTCOME

Mean scores for the Bayley mental scale, Bayley motor scale and academic scale of Developmental Profile II (IQ equivalent) are shown in table 1. These results are for the 212 infants seen by RM at 18 months after full term. There are no significant differences between the mean scores of children from control and treated groups for the whole cohort or for the babies of less than 30 weeks' gestation.

Developmental outcome is considerably influenced by social and demographic factors. To be certain that any minor imbalances were not biasing the results, the scores for all infants were entered into multiple regression analysis as dependent variables. Independent factors included in the models were treatment with surfactant and pre-existing factors at randomisation: sex, gestation, social class, and birth rank. The results confirmed that surfactant treatment was not significantly related to the Bayley mental scale, IQ equivalent, or Bayley motor scale in the whole group or in those above and below 30 weeks' gestation, whether or not children with neurological impairment were included.

In neurologically normal infants, five of 105 controls (5%) controls were diagnosed as mentally impaired (Bayley mental development index >70 in those tested by RM) compared with four of 103 treated infants (4%). The incidence in babies of less than 30 weeks' gestation was three of 38 controls (8%) and two of 47 treated babies (4%).

NORMAL SURVIVAL IN BABIES BORN AT LESS THAN 30 WEEKS' GESTATION

In the surviving children born before 30 weeks' gestation, in whom mortality was significantly reduced by prophylactic treatment with artificial surfactant (ALEC), treated children had no

Table 1 Mean (SD) developmental score for control and surfactant treated groups at 18 months after full term. Results are shown for all children, and subdivided at 30 weeks' gestation

	All infants		*Infants of <30 weeks' gestation*		*Infants of ≥30 weeks' gestation*	
	Controls (n=107)	*Surfactant (n=105)*	*Controls (n=73)*	*Surfactant (n=64)*	*Controls (n=34)*	*Surfactant (n=41)*
Bayley mental development index*	104 (21)	101 (18)	97 (20)	95 (19)	107 (20)	103 (17)
IQ equivalent	107 (18)	105 (19)	102 (17)	100 (19)	109 (19)	109 (18)
Bayley motor development index*	93 (16)	93 (15)	88 (19)	90 (15)	95 (14)	95 (14)

*Scores for children with neurological impairment are excluded.

Table 2 Outcome of infants of less than 30 weeks' gestation

	Control (n=61)	*Surfactant (n=61)*
Neonatal deaths	20	10
Late deaths	3	4
Neurological impairment	6	9
Mental impairment*	3	2
Hearing loss only*	4	1
Normal survivors	25 (41%)	35 (57%)

*Those who also had neurological impairment are not included here.

Table 3 Number (%) of problems encountered after leaving the neonatal unit (only those seen by RM are included)

	All infants		*Infants of <30 weeks' gestation*	
	Control (n=107)	*Surfactant (n=105)*	*Control (n=34)*	*Surfactant (n=41)*
Respiratory infections*	50 (47)	57 (54)	19 (56)	27 (66)
Hearing loss	9 (8)	4 (4)	6 (18)	2 (5)
Otitis media	41 (38)	45 (43)	17 (50)	20 (49)
Hospital admissions† for:				
Operations	13 (12)	14 (13)	7 (21)	7 (17)
Respiratory infections	9 (8)	10 (10)	4 (12)	5 (12)
All causes	44 (41)	46 (44)	21 (62)	19 (46)

Some children had more than one admission.
*The number of children with one or more infections diagnosed and treated by a doctor.
†The number of children who had one or more admissions.

increase in the incidence of physical or mental impairment, or of hearing loss. Table 2 shows the outcome for all infants under 30 weeks' gestation who were randomised in Cambridge. Follow up information was complete for this group. Of 61 children randomised to each group, there were 25 unimpaired survivors in the control group (41%), compared with 35 in the group treated with surfactant (57%). The 95% confidence interval for an improved rate of normal survival was −1 to 33/100.

OTHER MEDICAL PROBLEMS

The incidence of respiratory infections after discharge from the neonatal unit, episodes of otitis media, hospital admissions, and operations did not differ significantly between the control and treated groups (table 3). There were no other significant differences between the control and treated infants. Food intolerance was reported by the mothers in 13 of those treated with surfactant (11%), and 12 control infants (11%). Two mothers from each group suspected that their child was allergic to eggs (the artificial surfactant contains egg lecithin). Three cases of mild wheezing were reported in the treated group (3%), compared with six mild cases and one severe case (diagnosed as asthma) in the control infants (6%). Mild infantile eczema was seen in 13 treated (11%) and 11 control infants (10%).

Discussion

Three trials of this surfactant have shown reductions in neonatal mortality and the incidence of brain haemorrhages.[1–4] As such premature babies are at high risk of neurological or mental impairment, we were concerned that the increased survival of those very premature babies who were treated could lead to an increase in the number of impaired survivors.

Our data, like those of Vaucher *et al*[10] and Halliday *et al*,[11] have shown no increase in the rate of neurological or mental impairment in those treated with surfactant. In those children born before 30 weeks' gestation (the group showing the greatest improvement in survival after treatment) the number of normal survivors was greater in the treated (35 of 61) than the control (25 of 61) group (table 2). This strong trend was however, not significant (χ^2 3·29, p=0·07). Children in the treated group did not differ from controls in their later requirements for admission to hospital or treatment of respiratory or middle ear infections, nor in their incidence of allergic reactions.

Treatment of very premature infants with prophylactic artificial surfactant (ALEC), significantly improved neonatal mortality, with no increase in the rate of neurodevelopmental impairment, respiratory illness, or allergies.

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Sonographic and pathological features of callosal hypoplasia in non-ketotic hyperglycinaemia

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Abstract

A boy was born at 36 weeks' gestation weighing 2450 g. Though his Apgar score was 9 at birth, by the age of 48 hours he required artificial ventilation. He was deeply unconscious with complete lack of muscle tone, and non-ketotic hyperglycinaemia associated with secondary hypoplasia of the corpus callosum was confirmed by biochemical tests. The cranial ultrasound scan features correlated well with the neuropathological findings and may be helpful in the early detection of this incurable condition.

Non-ketotic hyperglycinaemia is an autosomal recessive disorder with a distinctive neonatal presentation comprising hypotonia and coma in the absence of obvious metabolic disturbance.[1 2] The primary pathology is dysmyelination of the brain and brain stem with associated microvacuolation.[2 3] The most obvious macroscopic effect is secondary hypoplasia of the corpus callosum that causes the lateral ventricles to assume a 'bat's wing' appearance.[2 3] Although the computed tomographic appearances of non-ketotic hyperglycinaemia have been described,[4] there appears to have been no report to date of the cranial ultrasound features.

Case report

A boy weighing 2450 g was born at 36 weeks' gestation to a 21 year old single, primiparous mother with a stable non-consanguinous union. He required no resuscitation at birth (Apgar score 9 at one minute), but was described as 'floppy' by the nursing staff and needed tube feeding and mask resuscitation. At 38 hours of age he developed progressively severe apnoeic episodes, and by 48 hours required mechanical ventilation. He was transferred to this hospital and on examination was deeply unconscious (with no dysmorphic features), did not respond to painful stimuli, and made no spontaneous effort. There was complete lack of muscle tone but tendon reflexes were brisk. There were no abnormal movements and no signs of raised intracranial pressure. Mechanical ventilation was easy and blood gas measurements remained within normal limits in 25% oxygen. He had a grade 2/6 systolic murmur over the left sternal edge. A clinical diagnosis of non-ketotic hyperglycinaemia was confirmed by the biochemical findings.

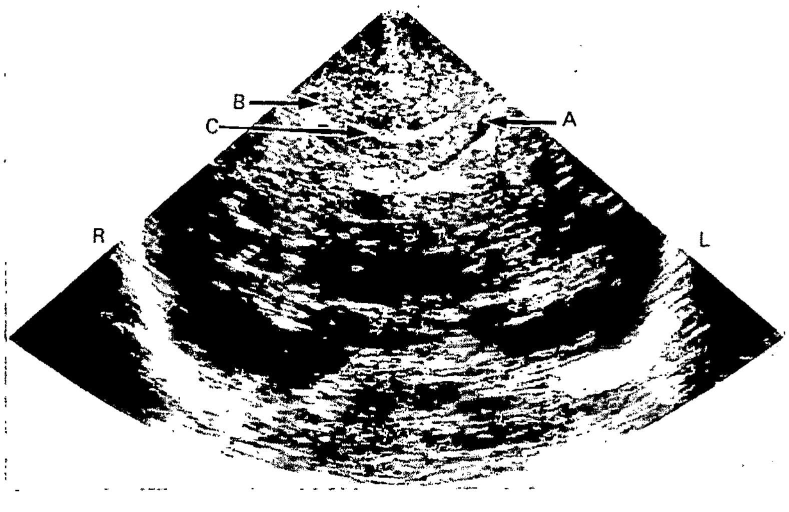

Figure 1 Coronal ultrasound scan of the brain showing widely separated and horizontal lateral ventricles (A) with areas of increased echogenicity containing small cysts in the periventricular white matter (B). (C=cingulate suclus, L=left, R=right.)

Plasma glycine concentration was 1676 mmol/l (normal 200–400), urinary glycine 1100 mmol/mmol creatinine (normal range 138–2100 mmol/mmol creatinine), and cerebrospinal fluid (uncontaminated with blood) glycine was 551 mmol/l (normal 10). Other amino acid concentrations, and the urinary organic acid profile, were normal. Plasma ammonia (as ammonium ion) was raised at 134 μmol/l. After the diagnosis was confirmed active treatment was withdrawn and he died at 9 days of age.

ULTRASONOGRAPHIC APPEARANCE

The cranial ultrasound scan done at the time of admission showed wide separation of the frontal horns and bodies of the lateral ventricles together with elongation of the foramen of Monro. The corpus callosum was difficult to identify (fig 1). In the sagittal view (fig 2) the corpus callosum could not be seen, although the cingulate gyrus was visible and the medial cerebral sulci had normal configuration. The third ventricle was normally situated. In some areas of the periventricular white matter there was increased echogenicity associated with tiny cysts.

NEUROPATHOLOGICAL FINDINGS

After they had been fixed in formalin, coronal sections were examined; they showed a small (2–3 mm) but visible hypoplastic corpus callosum (fig 3) beneath a dorsally displaced cingulate gyrus. Histological examination of paraffin embedded sections showed a small band of central myelinated fibre bundles in the mid por-

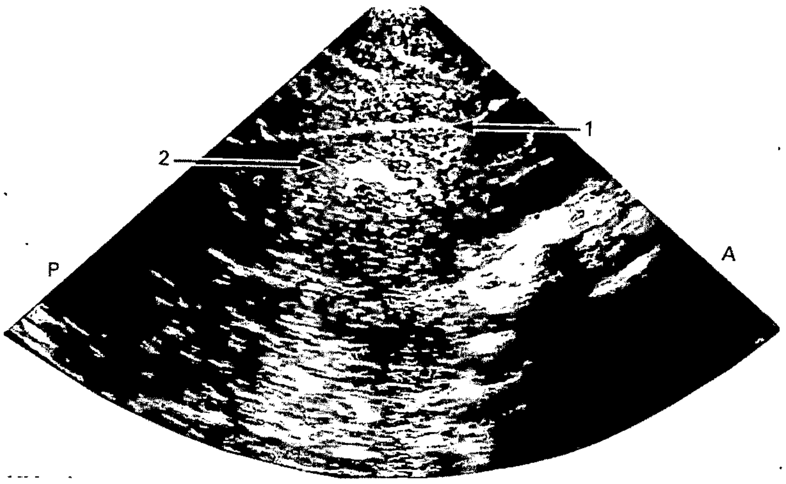

Figure 2 Midline sagittal ultrasound scan of the brain showing the cingulate sulcus (1) but no expected corpus callosum is visible in this area (2). (A=anterior, P=posterior.)

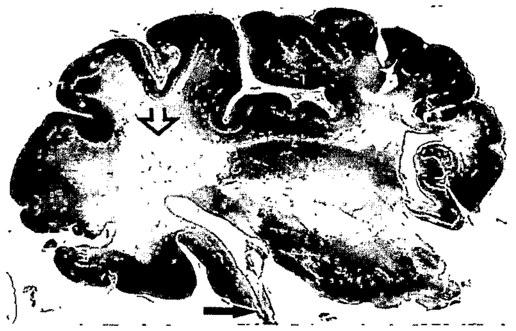

Figure 3 Right hemisphere coronal section (level of mamillary body) showing hypoplastic corpus callosum (solid arrow) and ventromedially displaced cingulate gyrus. Note that the fornix remains attached to the ventral (inferior) surface of the corpus callosum. Abnormalities of white matter are indicated (open arrow). (Formalin fixed, paraffin embedded 15 μ cresyl violet stained section about one and a half times actual size.)

tion of the corpus callosum, which confirmed callosal hypoplasia. The central hemispheric white matter showed focal rarefaction and hypocellularity in the periventricular regions. In this region ependyma in the lateral ventricular angles (and beneath the corpus callosum) was present as an incomplete layer, and where it was absent the ventricular wall consisted solely of white matter and glial tissue. In these areas small ependymal rosettes were present suggesting that the ependymal layer had been disrupted or non-existent for some time before death.

Discussion

This patient had the clinical, biochemical, and pathological features of neonatal non-ketotic hyperglycinaemia. The neuropathological examination showed extensive dysmyelination of the cerebral white matter and hypoplasia of the corpus callosum, confirming the features seen on ultrasound scanning. Intracranial haemorrhage has previously been described, and it is interesting that the two cases described by Holmqvist and Polberger were infants born at full term.[1]

Ultrasound comparison of corpus callosum hypoplasia and callosal agenesis

Feature	*Hypoplasia*	*Agenesis*
Distinctive features:		
Reactive dilatation of the occipital horns	Absent	Present
Dilatation and upward displacement of the third ventricle	Absent	Present
Cingulate gyrus	Present	Abnormally rotated
Arrangement of the medial cerebral sulci	Normal	Radial
Common features:		
Separation of the frontal horns and bodies of lateral ventricles	Present	Present
Narrow frontal horns	Present	Present
Elongation of the foramen of Monro	Present	Present

The ultrasonographic features of total and partial agenesis of the corpus callosum and its associated anomalies have been described in some detail,[5–8] but although the possibility of secondary hypoplasia of the corpus callosum has been discussed,[6] and cases have been described,[9] the ultrasonographic features have not been delineated.

In the table the features of hypoplasia and agenesis of the corpus callosum are compared. It is apparent that disruption of a corpus callosum that is already partly formed has different effects on brain development from complete aplasia, and that these differences can be detected by ultrasonography.

Neonatal non-ketotic hyperglycinaemia is a devastating disorder for which no effective treatment is available.[1 2] The presence of intracranial abnormalities at birth as seen in these and other patients suggests that any treatment will always have a limited success.[2 3] In spite of this early recognition of the disorder is important so that appropriate management and parental counselling can be offered. Many of these babies will have cranial ultrasound scans during their intitial assessment. Recognition of the potential importance of callosal hypoplasia may give the clue to the real nature of the underlying disorder.

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Red cell volume and cardiac output in anaemic preterm infants

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Abstract

To test the hypothesis that haemoglobin concentration is a poor predictor of benefit from transfusion in preterm infants, and that red cell volume is the most important indicator of anaemia, 24 preterm infants receiving red cell transfusions had red cell volume, haemoglobin concentration, and cardiac output measured before and after transfusion. Red cell volume was measured either using dilution of autologous fetal haemoglobin with donor adult haemoglobin, or with a new technique using biotin as a red cell label. The two techniques give similar results. Mean (SD) values before transfusion were 27·4 (13·3), and after transfusion 45·0 (13·7) ml/kg. Cardiac output was measured using imaging and Doppler ultrasonography, and fell with transfusion from mean 286 (121) to 251 (95·6) ml/kg/min. The red cell volume before transfusion correlated well with changes in cardiac output following transfusion, infants with a red cell volume before transfusion of less than 25 ml/kg showing a fall in cardiac output, and those with a red cell volume of greater than 25 ml/kg not showing a significant fall. There was no correlation between haemoglobin concentration, packed cell volume, or change in packed cell volume with changes in cardiac output after transfusion.

A red cell volume of 25 ml/kg seems to be critical in preterm infants with anaemia, and infants with values below this are those most likely to benefit from transfusion.

Blood transfusions are commonly used in the management of preterm infants yet indications for transfusion and volumes of blood used vary widely. Haemoglobin concentration before transfusion is a poor predictor of benefit from red cell transfusion,[1 2] and it has been suggested tht the red cell volume (synonymous with red cell mass) is theoretically a better guide to transfusion needs.[3–5] The relationship between red cell volume and haemoglobin concentration varies[4–7]; though some of this variation may be explained by differences in sampling sites for haemoglobin concentration, it may also be the result of differences in plasma volume among infants. Some infants have low red cell volumes, yet maintain a relatively normal haemoglobin concentration.[4–6] The association of decreasing blood volume and a decreasing red cell volume may partly explain this phenomenon.[3 6]

In anaemic infants the packed cell volume is low. This reduces the oxygen carrying capacity of the blood but is partly compensated for by reduced viscosity of the blood and consequent reduced resistance to blood flow; flow increases thus partly restoring oxygen delivery. It has been calculated that the optimal packed cell volume for oxygen transport is about 40%.[8] The effective reduction in peripheral vascular resistance should result in an increase in venous return and so in cardiac output.

An increased cardiac output has been found in anaemic adults[9] as well as in newborn infants,[10] and theoretically should also occur in anaemic infants.[3]

The purpose of this study was to investigate the value of measurements of red cell volume in predicting the need for, and benefit from, red cell transfusion defined by a fall in cardiac output.

Patients and methods

Cardiac output, red cell volume, haemoglobin concentration and packed cell volume were measured before and after red cell transfusions in 24 preterm infants without clinical evidence of patent ductus arteriosus. The decision to transfuse was made on clinical grounds independently of this study. Details of the infants are given in table 1.

Ten infants were ventilated, four were being given supplementary oxygen, and 10 were breathing air at the time of study. Measurements were made while the infants were either asleep or in a quiet but alert state, and being nursed in an incubator or cot, as clinically indicated. As far as possible subsequent measurements were made with the infants in a similar state on each occasion.

CALCULATION OF CARDIAC OUTPUT

Cardiac output was calculated with a Vingmed CFM 700 Duplex ultrasound scanner with a 5 MHz probe.[11] Spectral signals of aortic blood flow were recorded by continuous wave Doppler from the suprasternal site. Audio signals were later analysed on a Doptek spectrum analyser, which edits and calculates the mean blood flow

Table 1 Details of 24 infants (figures given are number of infants except where otherwise stated)

Gestation age (weeks):	
24–26	8
27–28	4
29–30	7
31–32	3
33–34	2
Mean (range) age at transfusion (days)	25 (2–90)
Mean (SD) birth weight (g)	1050 (26)
Mean (SD) weight at time of study (g)	1530 (66)

velocity. Aortic diameter was measured from the M mode echocardiogram through the aortic annulus using the trailing edge to leading edge method. The presence of a ductus arteriosus was defined using colour Doppler. If a ductus arteriosus was found the infants were excluded from later analysis because of uncertainties of the effect of transfusion on the size of the shunt.

The main limitation in the calculation of cardiac output is in the accurate measurement of aortic diameter.[11] To overcome this the percentage change in cardiac output (expressed as ml/kg/min) was calculated as follows:

$$\frac{\text{Output before transfusion}-\text{output after transfusion}}{\text{Output after transfusion}}\times 100$$

It was assumed that the aortic diameter measured at the annulus did not change appreciably in the 24 hours after transfusion, and this measurement was used on both occasions. The percentage change in cardiac output within an infant is therefore independent of the aortic diameter, thus enabling more accurate comparison among different infants.

Measurements were made before the blood transfusion and were repeated 12–24 hours afterwards.

MEASUREMENT OF RED CELL VOLUME

Red cell volume was measured by either diluting the fetal haemoglobin with donor adult haemoglobin[4] or labelling the red cells with a biotin tracer.[6] The former method could only be used if at least 20% fetal haemoglobin was present before the transfusion. This was possible in 20 infants. The concentration of fetal haemoglobin was accurately measured using an alkali denaturation technique. The biotin method for measuring red cell volume was used in four infants with low concentrations of fetal haemoglobin and also in six others at the same time as the dilution of fetal haemoglobin with donor adult haemoglobin as part of a previous study to confirm that the two methods gave similar results (table 2).

The technique for measuring red cell volume in preterm infants using biotin labelling of red cells has been described in detail previously.[6] Autologous red cells were labelled with long chain water soluble biotin (NHS-LC-biotin sulfosuccinimidyl 6-(biotinamide))(Hexanoate, Pierce Chemical Company) and reinjected. Blood samples were then taken from the infant at 10 and 30 minutes and combined with fluorescein-streptavidin (Amersham International), which allowed the proportion of positively labelled cells to be detected on a fluorescence activated cell sorter.

Haemoglobin concentration was measured in part of the blood sample taken for measurement of the red cell volume and was arterial if the infant had an indwelling arterial catheter, or venous if not.

Table 2 Simultaneous measurements of red cell volume (ml/kg) by biotin and dilution of fetal haemoglobin

Biotin	*Dilution of fetal haemoglobin*
28·0	30·5
26·1	27·2
20·3	20·0
13·1	16·0
19·6	19·3
16·4	15·4
Mean (SD) 20·6 (5·66)	21·4 (6·13)

BLOOD TRANSFUSIONS

Blood transfusions were given for clinical reasons such as blood loss caused by sampling or refractory anaemia of prematurity. About 30 ml/kg of packed red cells (20 ml/kg pure red cells) were given at each transfusion.

Results

The mean (SD) results of the simultaneous determination of red cell volume using biotin—20·6 (5·7)—and dilution of fetal haemoglobin—21·4 (6·1)—were similar (table 2). There was no significant difference between the results (paired *t* test, p>0·05).

The relationship between the red cell volume and haemoglobin concentration before transfusion is shown in fig 1 (r=0·663).

Cardiac output, red cell volume and haemoglobin concentration are shown in tables 3 and 4. The overall difference in the cardiac output was significantly different after transfusion when compared with the values before trans-

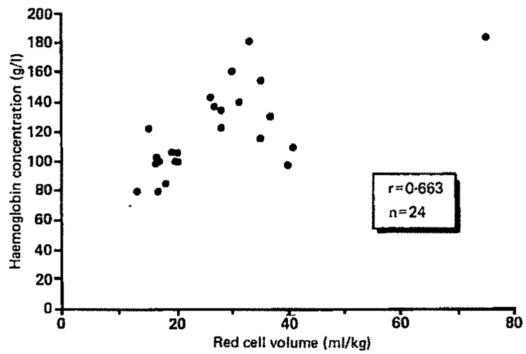

Figure 1 Association between red cell volume and haemoglobin concentration before transfusion.

Table 3 Mean (SD) values for cardiac output, red cell volume, and haemoglobin concentration in 24 infants

	Before transfusion	*After transfusion*
Red cell volume (ml/kg)	27·4 (13·3)	45·0 (13·7)
Haemoglobin concentration (g/l)	120 (29)	177 (23)
Cardiac output (ml/kg/min):		
All infants	286 (121)	251 (95·6)
Infants without patent ductus arteriosus (n=20)	281 (116)	240 (85·0)

Table 4 Mean (SEM) values for cardiac output, red cell volume, and haemoglobin concentration in 24 infants

	Before transfusion	*After transfusion*
Red cell volume (ml/kg)	27·4 (2·6)	45·0 (2·8)
Haemoglobin concentration (g/l)	120 (6)	177 (5)
Cardiac output (ml/kg/min):		
All infants	286 (24·7)	251 (19·5)
Infants without patent ductus arteriosus (n=20)	281 (25·9)	240 (19·0)

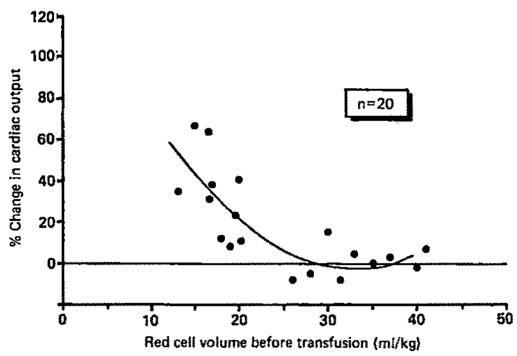

Figure 2 Association between red cell volume before transfusion and cardiac output before and after transfusion.

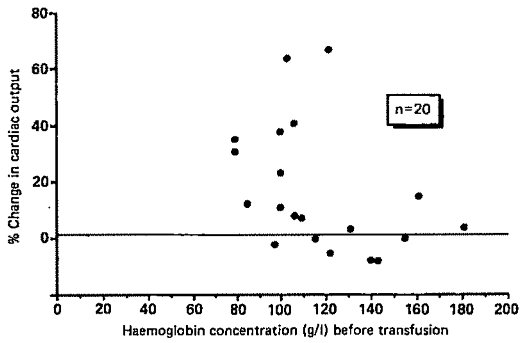

Figure 3 Association between haemoglobin concentration before transfusion and cardiac output before and after transfusion.

fusion (Student's *t* test for paired samples, $p<0{\cdot}05$).

Four of the 24 infants had patent ductus arteriosus shown on Doppler ultrasonography. Figure 2 shows the relationship between the percentage change in cardiac output and the red cell volume before transfusion for infants without patent ductus arteriosus. This relationship is expressed by the equation: percentage change in cardiac output=153−9·44×red cell volume+0·143 (red cell volume).^{2} The appropriate SE of the coefficients are 36, 2·9, and 0·054, and the *t* ratios 4·26, −3·22, and 2·64. R−sq=65·0%. The estimated standard deviation of the percentage change is 14·04. Clearly all terms in the relationship are significantly different from zero. It shows that those infants with a red cell volume of more than 25 ml/kg before transfusion did not have a significant fall in cardiac output, whereas those infants who had a red cell volume of less than 25 ml/kg before transfusion did have a significant fall in cardiac output. There was no significant relationship between the percentage change in cardiac output and haemoglobin concentration before transfusion (fig 3), (R−sq=15·5%). Similarly there was no significant relationship between percentage change in cardiac output and packed cell volume before transfusion (R−sq 15·1%), change in packed cell volume (R−sq 6·6%), or change in haemoglobin concentration (r−sq 4·1%).

Discussion

The ability to predict benefit from blood transfusion reliably would permit better prescribing of red cell transfusion, and would lead to a reduction in exposure to donors and hence the risks of transfusion transmitted infection. This study confirms the variable relationship between red cell volume and haemoglobin concentration, and this may partly explain why the haemoglobin concentration is a poor predictor of benefit from blood transfusion. Measurements of cardiac output and red cell volume were made before and after blood transfusion. In the analysis of the results, infants in whom patent ductus arteriosus was found either before, after, or both before and after (n=4), were excluded. Alverson *et al* found that infants with patent ductus arteriosus had increased cardiac output,[12] and it is not yet fully established what the effect of transfusion is on shunt size in these infants.

The mean cardiac output was significantly lowered after transfusion; others, however, have not found this change to be significant.[1] In the present study larger volumes of red cells were used (about 20 ml/kg) than in other studies, [10] and continuous wave Doppler was used in preference to pulsed Doppler. We have previously found continuous wave Doppler to be more reproducible than pulsed Doppler in calculating of cardiac output in newborn infants.[11]

Jones *et al* predicted a biological response from theoretical considerations of perfusion and packed cell volume, which would occur when the red cell volume fell to between 15 and 25 ml/kg.[3] This response could be an increase in cardiac output. In the 20 infants without evidence of patent ductus arteriosus the percentage change in cardiac output with transfusion was similar to the predicted biological response, and was significantly related to the red cell volume before transfusion. Those infants with a red cell volume of less than 25 ml/kg showed the greatest fall and those infants with red cell volume of greater than 25 showed no significant change. There was no significant association with haemoglobin concentration, packed cell volume, change in haemoglobin concentration, or change in packed cell volume.

It might have been expected that the change in cardiac output would have had a stronger association with the change in packed cell volume (fig 4) because of considerations of flow and blood viscosity. The lack of relationship

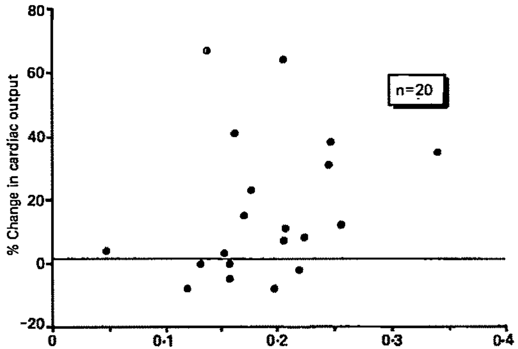

*Figure 4 Association between change in packed cell volume with transfusion and cardiac output before and after transfusion. *Packed cell volume after transfusion—packed cell volume before transfusion.*

found in this study may be the result of several factors. Firstly, the sampled packed cell volume may bear only a limited association to total body packed cell volume (red cell volume/total blood volume). Correction factors have been derived to correct for total body to venous packed cell volume ratios, but these are mean values for a number of newborn infants and considerable individual variation might be expected.[13 14] The relationship between total body and venous packed cell volume ratios have not been fully investigated in sick newborn infants, and the individual variation may be even greater. Secondly, total blood volume, which appears to be related to red cell volume,[3 6] may play an important part. There seems to be no evidence that plasma volume expands in anaemic infants,[3] and therefore in these infants with reduced red cell volume the effect of a reduction in blood volume and shunting to maintain flow to vital organs is superimposed on the effects of a reduced packed cell volume. Bifano *et al* found that albumin infusions given to anaemic infants relieved the apnoea that had been attributed to the anaemia, at least during the 24 hours after the infusion.[15] It seems likely, therefore, that some of the symptoms attributed to the anaemia of prematurity are the result of the reductions in blood volume found in anaemic infants.

This study clearly shows that changes in cardiac output associated with transfusion were significantly related to red cell volume but not to haemoglobin concentration or packed cell volume. Red cell volume may therefore be a better guide for decisions on when to transfuse and a better predictor of benefit from tranfusion.

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Use of peripheral vessels for exchange transfusion

T F Fok, L Y So, K W Leung, W Wong, C S Feng, S S Tsang

Abstract

During a five and a half year period, exchange transfusions were performed through the peripheral vessels in 201 of the 214 infants (94%) who required either double volume or partial plasma exchange transfusions. Peripheral vessel exchange transfusion is simple, practicable, and safe with few complications. Technical difficulties in catheterising the peripheral artery and vein may be overcome by using a 24 gauge catheter, which causes no more catheter induced haemolysis than standard umbilical catheters.

To avoid the complications of exchange transfusions done by the conventional 'push pull' technique through the umbilical vein,[1 2] an alternative technique using the peripheral vessels was successfully attempted in selected infants.[3–5] Since the opening of our neonatal unit in 1984 we have routinely used this technique on all infants requiring exchange transfusion. This report reviews our five and a half year experience with the technique in 201 infants.

Patients and methods

The case notes of all infants who received exchange transfusions between April 1984 and October 1989 were reviewed. During this period a total of 87 newborn infants with neonatal hyperbilirubinaemia received double volume exchange transfusions. During the same period partial exchange transfusions with plasma were carried out in 127 infants with polycythaemia. Attempts were made to carry out the exchange through a peripheral vessel in all except three premature infants who had indwelling umbilical arterial catheters already in place. The indication for exchange transfusion in infants born at full term was a serum bilirubin concentration of more than 340 µmol/l. In premature infants, exchange transfusions were carried out when the serum bilirubin concentrations were lower depending on the gestational ages and birth weights. Indications for partial exchange transfusions were venous packed cell volume of over 0·70 in any infant, and symptoms related to hyperviscosity such as respiratory distress, lethargy, and excessive jitteriness in infants with venous packed cell volumes of 0·60–0·70.

TECHNIQUE

A peripheral artery (radial or posterior tibial) was cannulated percutaneously with a 24 gauge Angiocath (Deseret Pharmaceutical) under aseptic conditions. Before each cannulation Allen's test was carried out to confirm the adequacy of collateral circulation of the cannulated limb.[6] The course of the artery was located either by palpation or by transillumination with a fibreoptic light source. The catheter was connected to a syringe and three way stopcock so that blood could be withdrawn and discarded intermittently. A 22 gauge or 24 gauge catheter of a similar make was inserted aseptically into a superficial vein of another limb for the infusion of donor blood or plasma. Prewarmed blood in citrate-phosphate-dextrose not more than three days old was used for all exchange transfusions. Withdrawal and replacement of blood (or plasma) were carried out simultaneously at a rate of 5–10 ml/min by two different operators. As a prophylactic measure to prevent clotting during exchange transfusions, the arterial catheter was flushed intermittently with 1 ml of heparinised saline (5 units/ml) after withdrawal of every 50 ml of blood.

All exchanges were carried out under radiant warmers. The general condition of the infants and the circulation of the catheterised limbs were closely observed. Heart rate, respiratory rate, and skin temperature were continuously monitored electronically. The arterial blood pressure of infants receiving exchange transfusion was measured intermittently through the arterial line, which was connected to a pressure transducer by means of a three way stopcock. The catheters were removed at the end of the procedure, unless the infant had severe haemolytic hyperbilirubinaemia and was expected to require repeat exchange transfusions, in which case the catheters were kept patent by continuous infusion of heparinised saline (1 unit/ml) at a rate of 1 ml/hour. Serum bilirubin concentration and packed cell volume were measured two hours after both exchange transfusion and partial exchange transfusion to determine the efficacy of the exchanges. Infants were observed in the neonatal unit for at least three days after the procedure.

EVALUATION OF CATHETER INDUCED HAEMOLYSIS

The safety of using 22 gauge catheters from the point of view of catheter related haemolysis has been reported previously.[3] As the narrower 24 gauge catheters were used in our patients, we compared the amount of haemolysis caused by these catheters with that caused by the standard 5 and 3·5 French gauge 'Argyle' umbilical vessel catheters (Sherwood Medical). Plasma

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Mean (SD) plasma haemoglobin concentrations of donor blood after infusion at three rates through a 24 gauge Angiocath, a 5 French gauge umbilical catheter and a 3·5 French gauge umbilical catheter (n=14 in each group)

Infusion rate (ml/min)	*Plasma haemoglobin concentration (g/l)*			*Analysis of variance*	
	24 gauge Angiocath	*5 French gauge umbilical catheter*	*3·5 French gauge umbilical catheter*	*F*	*p Value*
5	0·44 (0·08)	0·44 (0·08)	0·45 (0·11)	0·14	0·87
10	0·43 (0·07)	0·45 (0·10)	0·46 (0·10)	0·52	0·60
15	0·45 (0·08)	0·43 (0·08)	0·49 (0·09)	1·65	0·20

All values significantly greater than the mean (SD) concentration before infusion of 0·36 (0·05).

haemoglobin concentration estimated by the cyanmethaemoglobin method was used as the marker of haemolysis.[7] Fourteen blood samples were drawn from five units of eight day old blood in citrate-phosphate-dextrose with known plasma haemoglobin concentrations. Each sample was then manually infused at three different infusion rates—5, 10, and 15 ml/minute—through each of these catheters. The plasma haemoglobin concentrations after infusion through each catheter at each rate were measured and compared with the preinfusion concentration by Student's *t* test, and also among themselves by analysis of variance.

Results

During the study period peripheral vessel exchange was successfully carried out in 79 infants requiring exchange transfusion and 122 infants requiring partial exchange transfusion, giving an overall success rate of 95% (201 of 211). We had to resort to the umbilical route in five infants born at full term in whom cannulation of a peripheral artery was unsuccessful. Among those who had their exchanges by the peripheral route, eight (five receiving exchange transfusions and three receiving partial exchange transfusions) presented with respiratory distress and were given antibiotic prophylaxis before the exchange.

The group of infants receiving peripheral exchange transfusion had gestational ages ranging from 30·1 to 41·2 weeks, and birth weights ranging from 1370 to 4100 g. Their serum bilirubin concentrations ranged from 299 to 459 µmol/l before exchange transfusion, and 128 to 240 µmol/l two hours afterwards. The mean drop in serum bilirubin concentration was 163 µmol/l (range 110–205), or 45% of the concentration before exchange. The volume of donor blood exchanged varied from 300 to 600 ml depending on the size of the infant. Most of the exchange transfusions were completed within 90 minutes (range 45–175, mean 82). One infant with glucose 6–phosphate dehydrogenase deficiency had three exchanges; two other infants with the same deficiency, and two with ABO incompatibility, required two exchanges each. The same arterial lines were used for the repeat exchanges.

The gestational ages and birth weights of the infants who received partial exchange transfusion by the peripheral route were 33·1–42 weeks, and 1720–4620 g, respectively. Packed cell volumes before partial exchange transfusion ranged from 0·63 to 0·82. All babies showed a satisfactory drop in packed cell volume of 0·07 to 0·25 (mean 0·19) to 0·49 to 0·64 two hours after the exchange. As the partial exchange transfusions required smaller volumes of blood (24–100 ml), the time required for transfusion was only 5–30 minutes (mean 16).

There were no deaths, and the few complications mainly affected those who had had exchange transfusions. The arterial catheters became blocked in four infants and dislodged in two. Subcutaneous extravasation of infused blood occurred at the venous side in two infants. In all eight infants the exchange was completed after successful reinsertion of the catheters. One infant developed *Escherichia coli* septicaemia associated with a urinary tract infection two days after exchange transfusion. This infant was subsequently found to have bilateral grade III vesicoureteric reflux that later resulted in recurrent urinary tract infections.

The retrospective nature of this study did not allow us to analyse in detail the cardiorespiratory events that took place during the exchanges. We can only note that there were no appreciable untoward changes in heart rate, respiratory rate, and blood pressure during any of the procedures. Ischaemia of the catheterised limbs was not seen. All infants continued to be fed according to their routine schedule before and after the procedure. None developed any food intolerance or other abdominal complaints.

Results of the in vitro experiment on catheter induced haemolysis are shown in the table. We had used relatively old blood in the experiment, which should be more vulnerable to catheter induced mechanical damage. At each infusion rate all three catheters produced significant haemolysis as shown by an increase in plasma haemoglobin concentrations in the blood samples taken after infusion. There was, however, no significant difference in the plasma haemoglobin concentrations after infusion among the three types of catheter at each infusion rate, showing that the 24 gauge Angiocath did not cause more haemolysis than either the 5 or 3·5 French gauge umbilical catheters.

Discussion

Exchange transfusions using the umbilical vein or artery are associated with complications such as haemoperitoneum,[8] intestinal perforation,[9] necrotising enterocolitis,[8 10] air embolism,[2] portal hypertension,[8 11] and introduction of infection from the umbilical stump.[12] Gastrointestinal complications seem particularly common in polycythaemic infants treated with partial exchange transfusion by the umbilical route.[13] Fluctuations in circulatory volume resulting from the conventional 'push-pull' technique have been associated with cardiovascular complications including sudden cardiac arrest, pulmonary oedema, bradycardia,[15] and changes in cerebral blood flow and intracranial pressure.[16] A double catheter technique using both the umbilical artery and vein for simultaneous withdrawal and replacement of blood has been described,[17] but this method carries the risks of catheterisation of both the umbilical artery and the umbilical vein.

The peripheral vessels have been used as an alternative route for exchange transfusions in selected infants by previous workers.[3–5] Our experience gives further evidence that the technique can be used routinely in most babies who require double volume or partial plasma exchange transfusion. Of our exchanges, 95% were successfully carried out by the peripheral route resulting in satisfactory drops in serum bilirubin concentrations in the hyperbilirubinaemic infants, and packed cell volumes in the polycythaemic infants. Apart from the six infants who required recatheterisation of the artery, none of our patients developed any complications associated with the procedure. The *E coli* septicaemia that developed in one of the infants was probably related more to his urinary tract abnormality than to the exchange transfusion.

By avoiding the umbilical vessels, the technique should theoretically be associated with a reduction in risks of infection and intra-abdominal complications. Removal and replacement of blood with blood or plasma take place simultaneously, thus minimising cardiovascular changes caused by fluctuations in circulatory volume. Throughout the procedure only the limbs need to be exposed and immobilised, and temperature control becomes less of a problem as the infants' clothing need not be removed. As gastrointestinal complications are not a problem, fasting of the infants before and after the procedure is unnecessary. Unlike the umbilical route, the peripheral route can be used in infants of any age, including those in whom the umbilicus has healed.

Success in carrying out peripheral vessel exchange transfusions depends largely on successful placement of the arterial catheter, which may be technically difficult especially in vigorous infants born at full term with relatively thick skin. In our experience, peripheral arterial cannulation with the 24 gauge catheter is much easier than with the 22 gauge catheter,[3, 4] and is more secure than with the scalp vein needle that was used by previous workers.[5] Despite its narrower lumen, we did not encounter any difficulties in withdrawal of blood and most of our double volume exchange transfusions could be completed within a reasonable time.

On the venous side we have used both the 22 gauge and 24 gauge catheters for plasma or blood replacement. The safety in using the 22 gauge catheter for blood infusions has been shown by Campbell and Stewart.[3] Our in vitro study on catheter induced haemolysis shows that the 24 gauge catheter does not cause any more haemolysis than either the 5 or the 3·5 French gauge umbilical catheter.

Despite its practical and theoretical advantages, exchange transfusion though the peripheral vessels does not seem to have gained much acceptance by paediatricians since it was first described. Our experience shows that it is simple, practicable, and safe, with few complications. In the light of the existing evidence that both sick premature and healthy infants born at full term may develop complications when exchange transfusion is carried out by the umbilical route,[12] we believe that using the peripheral route is one way to minimise iatrogenic risks and should be considered for all infants requiring exchange transfusion.

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Illuminance of neonatal units

J Robinson, M J Moseley, A R Fielder

Abstract

We have measured the illuminance (brightness) of seven neonatal units during both the day and the night. When the units were lit solely by fluorescent tubes the mean illuminance was 348 lux (range 192–690). During the day the mean illuminance was 470 lux (range 236–905). The high dependency regions in four of the seven units were significantly brighter than the corresponding low dependency nurseries at all times. In two of these units there is a policy of reducing the amount of artificial light in the low dependency areas at night, and in these the normal mean illuminance was 50 lux. We have measured the general levels of illumination to which a neonate might be exposed; the ocular exposure to light of a neonate depends, however, on both physical and biological factors and more research is required before an accurate estimate can be made.

Preterm infants are cared for in neonatal units that are usually lit by a combination of natural and artificial light, the latter provided by fluorescent tubes. The guidelines governing the lighting of these units are set by the Chartered Institute of Building Services in the United Kingdom.[1] These guidelines state that the lighting of all interiors should fulfil three functions. Firstly, it should ensure the safety of the people in the interior; secondly, it should facilitate the performance of visual tasks; and, finally, it should create an appropriate visual environment. For neonatal units the 1989 recommendations for lighting and luminaires (plastic shields in front of fluorescent tubes) were:

> 'In cot areas, the lighting and luminaires should be positioned as far away from the cots as practicable to avoid direct glare into the cots. Additional luminaires may be required over the cots to provide higher illuminance depending on the width and shape of the room and the cot layout.
>
> Blinds should be provided in areas where day-lighting from side windows or roof lights could cause glare to babies suffering from retinopathy of prematurity.
>
> In incubator areas, higher levels of illuminance are required for the examination and care of babies and this should be provided by low glare luminaires'.[2]

These guidelines also define the mean illumination of the areas within a neonatal unit. Illuminance levels of 50–100 lux for low dependency and 1000 lux for the high dependency regions are recommended. Between the hours of 2000 and 0800 (night) the guidelines suggest that the mean illumination should be reduced to 5 lux. It is not clear whether this applies to both high and low dependency regions.

Contrary to the guidelines, most neonatal units are usually lit both day and night, with no cyclic variations in intensity. Surprisingly, the levels of light to which preterm neonates are exposed are one of few environmental variables which are not monitored. Moseley and Fielder have recently reviewed the literature about the potential toxicity of light to the eyes of neonates, and noted that there is little data on the exposure to light of babies undergoing treatment in neonatal units.[3]

Abramov *et al* showed that there were deficits in both photopic contrast sensitivity (a reduction of 0·2–0·3 log units) and photoreceptor function in a group of children tested at the age of 7 years, who had been born preterm and had been exposed to continuous illumination as neonates.[4] The colour vision deficits resembled those of tritanopia, suggesting damage to the short wavelength (blue) cones. Glass *et al* reported a reduction in the incidence of retinopathy of prematurity among a group of neonates exposed to reduced lighting.[5] This study has been criticised on several grounds: it was sequential and infants were not randomised into clinical study groups; the light reduction was achieved by placing a filter over the incubators; the method was not accurately described, and the observers were aware of which babies were having which treatment.[6] Others studies have suggested that continuous exposure to bright light affects sleep states[7] and hormonal biorhythms,[8] and may increase the incidence of patent ductus arteriosus.[9 10] The absence of day and night cycles may be partly responsible for the delayed onset of periodicities and sleeping problems in preterm neonates.[11]

During the past two decades light intensities recorded in hospital neonatal units have increased by five to tenfold. In 1969 Giunta and Rath reported mean illuminances of 10 foot candles (~100 lux) in a standard dim nursery environment in England,[12] and by the 1980s levels were reported ranging from 200 lux in England,[11] through 630 lux in Australia,[13] to a peak of 10^4 lux in a neonatal unit in the United States, where daylight supplemented the artificial illumination.[14] No study has been carried out to examine specifically the illumination of neonatal units in the United Kingdom or the variations between high and low dependency regions of neonatal units.

Clearly, before considering the possible effects of light on the developing eye or visual system, a knowledge of the light dose received by neonates (the retinal irradiance) is necessary. In this study we have measured the illuminance in seven neonatal units in central England, both at

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night and during the day, to obtain data on the general levels of illumination to which a neonate may be exposed.

Methods

INSTRUMENTS

Illuminance was measured with a Macam Digital Photometer, Model L103. The sensor in the detector head unit is a large area photodiode that has long term stability and is not damged by sudden exposure to high levels of light. Cosine correction was used in the diffuser to simulate an ideal 180° field of view detector. A Commission Internationale d'Eclairage (CIE) photopic filter was fitted in front of the diode to give a spectral response similar to that of the human eye.

All measurements of lighting contain several sources of error that may accumulate to give a possible uncertainty in the measurement, which may often exceed 20%. In this study the Macam photometer was calibrated against a standard that was traceable to the National Bureau Standard (USA) with an accuracy of 5%; the photometer itself is only accurate to 10%. Further uncertainty in the measurement is introduced by the number of samples taken.[1]

SURVEY OF NEONATAL UNITS FOR POSITIONING OF MEASUREMENT POINTS

A lighting survey to measure the average illuminance of each neonatal unit was carried out. In field surveys of lighting the number of measurement points is determined by the 'room index', which is calculated as follows: room index=$L \times B/[H_m(L+B)]$ where L=length of room (m), B=breadth of room (m), and H_m= height from luminaire to working plane (m).

The minimum number of measurement points necessary to give an error of less than 10% in the estimate of mean illuminance in a room is then obtained from the Chartered Institute of Building Services guidelines for field surveys—that is, for a room index <1 and an error of $<10\%$ four measurement points are required, similar figures for room indices of 1–2, 2–3, and ≥ 3 are 9, 16, and 25. As we required an error of less than 5% the number of data measurement points was doubled.[2]

An accurate scale plan of each neonatal unit was prepared. The room indices for each nursery in the seven neonatal units were then calculated using the height above the floor of the mattress in the cot or isolette as the working plane. Each room was then divided into the correct number of segments and the illuminance measured at the centre of each segment, which was as nearly square as possible. The measurement positions were then plotted on to the floor plans at the centre of each segment.

TIMING OF MEASUREMENTS

Illuminance levels were surveyed under three lighting conditions in each unit. Firstly, at night when the units were lit solely by artificial illumination. Secondly, on dull overcast or rainy days, and finally on dry and bright or sunny days. All measurements were made between February and April 1989.

Artificial lighting only

Each of the seven neonatal units were visited at night on a single occasion. During this visit the ambient lighting was surveyed throughout the unit with the lighting set as normal by the nursing staff. In addition, in units 5 and 6, (which have a policy of dimming the lights in the low dependency regions between 2000 and 0700) measurements were made both with the fluorescent lamps turned on and under the dimmed lighting.

Total illuminance during the day

The units were visited on four occasions separated by intervals of about one week. The survey was carried out once during each visit, either in the morning or the afternoon. If the survey was carried out in the morning on the first visit, the second survey was made in the afternoon.

Timing of measurements: effect on readings

Repeated measurements of illuminance were made at hourly intervals between 0700 and 1800 in one neonatal unit. This was to provide an estimate of the variability in illuminance due to timing of the surveys on the measured values.

All illuminance data were obtained at the approximate height of the upper surface of the infant in a cot or isolette, as close to the exact position of the infant as possible (table 1).

METHOD OF ANALYSIS

The mean, median, and range of illuminances were calculated in three ways: firstly, for each unit as a whole; secondly, with the high and low dependency regions considered separately; and, finally, for every room in each of the seven neonatal units surveyed. The uniformity ratios (defined as minimum/mean, and minimum/maximum measurements) were calculated for each room, and the results compared with the Chartered Institute of Building Services guidelines.

Analysis of variance was used to compare the mean illumination for the units studied, both when each unit was considered as a whole, and after subdividing the units into high and low dependency regions as separate blocks.

Results

The uniformity ratio, the minimum divided by the maximum recorded light levels (UR=min/max), was calculated for each room studied. Values of greater than 0·79 were obtained for every room irrespective of time or day of measurement.

Table 1 Selected radiometric and photometric quantities

Quantity	*Units*
Irradiance (radiant power incident upon a surface/unit area of surface)	Watts/cm^2
Spectral irradiance (irradiance in a narrow wavelength band centred at a specific wavelength)	Watts/cm^2/nm
Illuminance (photometric analogue of irradiance weighted for the visual response of the eye)	Lux

Table 2 Variations in mean illuminance with weather conditions. The mean (SEM) unit of illumination is shown for each of the seven neonatal units studied on dry, sunny days; dull, overcast or wet days, and at night

Neonatal unit	*Dry and sunny*	*Dull and overcast and rainy*	*Mean illuminance at night (2000 to 0800)*		
			Throughout the unit	*High dependency area*	*Low dependency area*
1	288 (17·2) (n=188)	443 (13·6) (n=188)	301 (15·4) (n=94)	461 (22·2) (n=34)	222 (6·5) (n=60)
2	752 (35·3) (n=94)	905 (46·1) (n=94)	690 (42·6) (n=47)	900 (42·8) (n=16)	452 (32·2) (n=31)
3	288 (16·8) (n=90)	383 (14·6) (n=90)	192 (17·4) (n=45)	227 (19·0) (n=16)	183 (21·1) (n=29)
4	369 (15·2) (n=108)	452 (20·9) (n=108)	278 (19·2) (n=54)	365 (5·2) (n=36)	189 (14·5) (n=18)
5	625 (5·8) (n=114)	837 (14·9) (n=114)	225 (6·1) (n=57)	255 (11·3) (n=25)	215/50* (6·5) (n=32)
6	358 (33·6) (n=112)	399 (25·7) (n=112)	560 (23·2) (n=56)	691 (12·7) (n=25)	233/50* (6·5) (n=32)
7	236 (10·1) (n=102)	246 (14·6) (n=102)	193 (11·9) (n=51)	210 (14·5) (n=27)	141 (8·5) (n=24)
Total mean	417	524	348	444	233

*The amount of light with the fluorescent lights switched off; n=the number of measurement points.

ARTIFICIAL LIGHTING ONLY

The contribution of the artificial lighting to the total illuminance in the units was assessed by visiting each unit during the night when the level of ambient (natural) illumination was minimal.

Mean (SEM) illuminance for all units was 348 (74) lux. Significant differences were found between the seven units studied, however, the mean for the individual units ranging from 192 lux in unit 3 to 690 lux in unit 2 ($p<0·001$) (table 2). When the data from each special care baby unit were divided into high and low dependency regions these differences became more apparent. In general the units were uniformly bright or dim, with the most recently opened having the highest mean unit illuminances. The high dependency nurseries of units 1, 2, 4, and 6 were significantly brighter than their corresponding low dependency nurseries (table 2). Similar differences among units were found in the illumination of the low dependency nurseries. With the exception of units 5 and 6, the data in table 2 represent the normal minimum night time illuminance. Both these units have a policy of reducing the levels of light and noise in the low dependency nurseries whenever possible. When a second survey was carried out at these units, the mean illuminance in the low dependency nurseries was about 50 lux.

TOTAL ILLUMINANCE DURING THE DAY

The mean illuminance of the neonatal units when calculated from measurements taken during the day (0700 to 2000) was 470 lux (range 236–905). Two series of illuminance measurements were made, firstly when the weather conditions outside were either dry and bright (mean (SEM) 524 (94) lux), or secondly, when it was wet or cloudy, that is greater than three quarters of the sky covered with low cloud (mean (SEM) 417 (74) lux) (table 2). Each unit was visited on four separate occasions, twice on bright sunny days and twice when it was dull, overcast, or raining. Except for unit 7, which has no external windows, the ambient illumination caused a significant rise in the mean level of neonatal unit illumination (table 2). It was noted that the nursing staff usually drew the curtains (if present) to reduce the level of light falling on those babies directly facing a window on bright days. In units 1, 3, 4, and 6, some of the lights were switched off routinely on bright days by the nursing staff.

VARIATION IN ILLUMINANCE DURING THE DAY

Repeated measurements of illuminance were made in unit 1. Measurements were made at hourly intervals between 0700 and 1800. No significant variations were found between the mean unit illuminance throughout the day. The uniformity ratio (min/max) fell to 0·65 (below the recommended value of 0·8) because of high illuminance readings at measurement positions near the windows.

OTHER GENERAL INFORMATION

All study hospitals used fluorescent lights in the units as the primary source of illumination. Plastic shields (luminaires) were located in front of the fluorescent tubes in all the neonatal units. At night the artificial illumination was said to be maintained at the same level as during the daytime hours, except for two centres in which it was reduced to roughly one tenth (2–3 lux). In all the units, however, the nursing staff would often reduce the number of fluorescent tubes switched on if the monitoring of the babies would not be impaired.

Discussion

The data obtained from this survey of neonatal units show the levels of illumination to which preterm neonates were exposed during their inpatient stay in any of the seven units studied.

The mean illuminance at night (2000 to 0800) was 348 lux (range 192–690). Illuminance measurements made at night may be considered to be the minimum to which preterm neonates will be exposed during their stay in a neonatal unit. But in the case of units 5 and 6 these values are potentially misleading because the lights were normally dimmed to about 50 lux in the low dependency nurseries between 2000 and 0700 hours each day.[11]

The total mean illuminance of the neonatal units when measured during the day (0700 to 2000) was 470 lux (range 236–905). The levels of illumination for unit 6, obtained during the day were similar to estimates made previously.^{11} The range of mean unit illuminances (236–905 lux) was similar to that previously noted by Landry *et al* for neonatal units in the United States (285–1485 lux).^{15} We did not, however, record any light levels in the region of 10^{4} lux—a value quoted by Hamer *et al* for a unit in which daylight could supplement the artificial light sources.^{14}

The interunit variability that we found seems partly to reflect the age of the neonatal unit—the newest, which opened in 1988, had the highest level of artificial illumination and the dimmest was the oldest unit studied.

All high dependency nurseries were illuminated throughout the 24 hour period and thus showed little variation and were brighter than their corresponding low dependency nurseries (table 2). The illuminance in the low dependency rooms was cycled routinely in units 5 and 6 at the time of the survey.^{11} The reduction in illumination between high and low dependency nurseries probably reflects the improving health of the neonates, as less intense monitoring is required for these babies.

The effect of external weather conditions was studied briefly and our results suggest that additional ambient light entering the units through windows can make an important contribution to the amount of light in the units (table 2). Infant positions with high illuminance values, when compared with the averages, were located near outside windows, an effect that has been noted previously.$^{15\ 16}$ The data recorded from points not in direct line with the windows, however, were similar, and independent of external weather conditions.

Our results merely report the illumination levels at the time of the survey. The total light exposure of an infant during treatment in a neonatal unit is dependent on many factors. For example, babies are moved from high to low dependency nurseries as their conditions improve. Babies may also be moved round randomly within a nursery depending on the number of infants in each region at the time. Thus a baby could be located at a point associated with a high illuminance at one stage and then be relocated at another position associated with low illuminance.

The light dose to the eyes of preterm neonates is dependent on many physical and environmental variables. These include the position of the infant in the neonatal unit; whether he or she is in the high or low dependency region; the position of the cot, isolette, or incubator in the nursery in relation to outside windows; external weather conditions; the use of eyeshields; the baby's head position, and the the possibility of shading from equipment near the cot or incubator.

Most of the lighting comes from overhead fluorescent tubes, but in addition neonates are exposed to other lights. These include a variety of warming and procedure lights (their eyes may not necessarily be shielded), and exposure to phototherapy lights for the treatment of neonatal jaundice during which the eyes are covered with patches, which have a tendency to slip.^{17} Also, because of the risk of developing retinopathy of prematurity, preterm infants are often examined ophthalmoscopically while they are in the neonatal unit. The instrument normally used is the binocular indirect ophthalmoscope, with a maximum intensity of light about one log unit greater than that needed for 100% rhodopsin bleach^{18}; Noell found that a 10% bleach is sufficient to damage the photoreceptors. However, the infant's retinas will usually be exposed for less than 60 seconds, and generally as the setting used is not maximal, the potential hazard is less.

The total amount of light reaching the eyes of preterm babies while they are in the neonatal unit must be greater than that received by a fetus, but before any relation between light exposure and any ocular pathophysiology is suggested an accurate estimate of the quantity of light actually reaching the eye or retina must be made. To obtain such estimates a knowledge of both physical or environmental (for example, illuminance, area of luminaires, and shading effects) and physiological factors (for example, eyelid opening, transmission of light through the eyelid, pupil area, and transmission of light through the optic media) is required. This study is part of a larger project examining each of these factors and their influence on the ocular light dose.

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Atrial natriuretic factor in hydrops fetalis caused by Rh isoimmunisation

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Abstract

Plasma concentrations of atrial natriuretic factor were determined by radioimmunoassay in 16 human fetuses of between 19 and 38 weeks' gestation. Fifteen fetuses had varying degrees of anaemia as a result of Rh isoimmunisation, and one fetus was normal. Eight fetuses had ultrasonographic evidence of severe hydrops fetalis and an additional three fetuses had mild hydrops. Severely hydropic fetuses were more anaemic and immature than those with mild or no hydrops. Among fetuses from which samples were taken before in utero transfusion, concentrations of atrial natriuretic factor were higher in those with severe hydrops than in the other groups. An inverse relationship between the haemoglobin concentration and that of atrial natriuretic factor was found. In four fetuses in which severe hydrops resolved after intravascular transfusions in utero, there were significant decreases in plasma atrial natriuretic factor concentrations; in the fifth fetus the decrease was less pronounced.

Raised concentrations of atrial natriuretic factor in fetuses with severe anaemia and hydrops may be the result of atrial natriuretic factor release induced by hypoxia.

Several investigators have shown that human and animal fetuses synthesise and secrete atrial natriuretic factor into the circulation in response to various stimuli.[1–3] Receptors for atrial natriuretic factor have been found in normal human and sheep placentas, which suggests that this peptide has a role in fetal fluid and electrolyte homoeostasis.[4 5] In sheep, concentrations of atrial natriuretic factor are higher in lamb fetuses than in the ewes,[6 7] and non-immune fetal hydrops caused by atrial pacing increased circulating concentrations of atrial natriuretic factor.[7] Robillard and Weiner recently reported that acute volume expansion in human fetuses with immune haemolytic anaemia also resulted in increased concentrations of atrial natriuretic factor.[1] In their study the highest concentration was in a fetus with severe anaemia and hydrops, and the concentrations decreased as the hydrops resolved. We have also reported similar preliminary findings.[8]

The objectives of this study were to determine whether human fetuses with varying degrees of hydrops fetalis and anaemia as a result of Rh isoimmunisation have increased concentrations of atrial natriuretic factor, and if those concentrations change after resolution of the hydrops by intravascular transfusion in utero.[9]

Material and methods

Umbilical venous blood samples were obtained from human fetuses about to undergo intravascular exchange transfusion in utero for severe anaemia caused by Rh isoimmunisation. This was done using an ultrasound guided percutaneous needle as previously described.[9] All samples were taken before the start of the transfusion. Packed cell volume and haemoglobin concentration were measured with an automated cell counter.

Blood samples for plasma atrial natriuretic factor concentrations were collected in polypropylene tubes with heparin (1000 U/ml) and aprotinin (20 KIU/ml). After centrifugation plasma samples were stored at −80°C until analysis. Plasma atrial natriuretic factor concentrations were measured by radioimmunoassay (Peninsula Laboratories) using a previously described method.[10] The interassay variability was 12·7% and intra-assay variation 6·1%. The recovery in all assays was over 80%.

Assessment of gestational age was based on last menstrual period and early ultrasound examination. Hydrops fetalis was diagnosed by ultrasound and was graded as follows: (i) absent, no evidence of ascites, pleural or pericardial effusion, or subcutaneous oedema; (ii) mild, a small amount of fluid in only one place (usually ascites); and (iii) severe, large amount of fluid in two or more places.

Statistical analysis was by one way and repeated measures analysis of variance (ANOVA), the Mann-Whitney U test and linear regression. When the data were skewed, they were analysed after logarithmic transformation. A probability of <0.05 was considered significant. All values are expressed as mean (SD).

Results

The study population comprised 15 fetuses with Rh isoimmunisation and one normal fetus who had samples taken at 38 weeks' gestation for determination of platelet count because of maternal immune thrombocytopenia. This fetus was normal on physical examination, and had a normal platelet count at delivery; the data were used not as a control but rather as an approximation of atrial natriuretic factor concentrations associated with normal haemoglobin concentrations. Blood was obtained from nine fetuses before they had received any transfusion in utero, whereas the other seven had had one or

Mean (SD) gestational ages and laboratory values in study group

	Fetuses without hydrops (n=5)	*Fetuses with hydrops*		*p Value**
		Mild (n=3)	*Severe (n=8)*	
Gestational age (weeks)	33 (3)	31 (3)	25 (4)	<0·01
Packed cell volume	0·27 (0·11)	0·20 (0·038)	0·12 (0·056)	<0·02
Haemoglobin (g/l)	89 (35)	64 (16)	39 (19)	<0·02
Atrial natriuretic factor (pg/ml)	73·9 (39·1)	75·4 (42·6)	576·5 (931·8)	0·1

*Those with severe hydrops compared with the other two groups by ANOVA.

more transfusions in the weeks before samples were taken.

Eleven fetuses had ultrasonographic evidence of hydrops, and in eight of them it was severe (table). The remaining fetuses had no evidence of hydrops. Those with mild or no hydrops were more mature than those in which it was severe (p<0·01 by one way ANOVA). All fetuses with Rh isoimmunisation had packed cell volumes of less than 0·34 and haemoglobin concentrations below 110 g/l. Those fetuses with evidence of severe hydrops were significantly more anaemic than those in the other groups (p<0·01, one way ANOVA). Most fetuses with severe hydrops had higher concentrations of atrial natriuretic factor than fetuses with either no or mild hydrops, but because of the large variability this difference was not significant (p=0·1, Mann-Whitney test). When only the atrial natriuretic factor concentrations of fetuses from whom samples were taken before they received any transfusion in utero were analysed, however, those with severe hydrops (n=6) had significantly higher concentrations (755·2 (1030·6) pg/ml, range 126–2796) than fetuses with no or mild (n=3) hydrops (70·9 (45·8) pg/ml, range 39·9–123·6) (p<0·05, Mann-Whitney U test).

An inverse relationship was found between haemoglobin and atrial natriuretic factor concentrations by using linear regression after logarithmic transformation (y=2·68−9·85e−2x, p<0·02) (fig 1). This association was also present when the analysis included only atrial natriuretic factor concentrations in samples taken before exchange transfusion in utero (y=2·88−9·58e−2x, r^2=0·51, p<0·05).

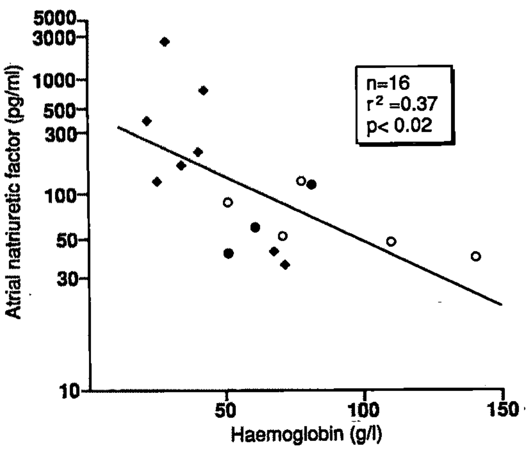

Figure 1 Relationship between haemoglobin concentration and plasma atrial natriuretic factor in all fetuses. No hydrops (○), mild hydrops (●), and severe hydrops (♦). The vertical axis represents actual values plotted on a semilogarithmic scale.

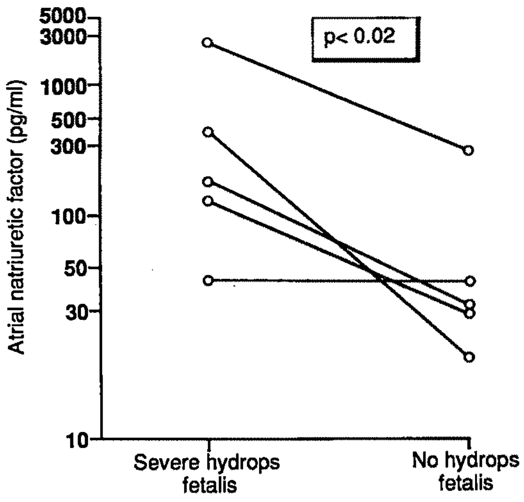

Figure 2 Plasma atrial natriuretic factor in fetuses sampled sequentially during severe hydrops and after complete resolution. The vertical axis represents actual values plotted on a semilogarithmic scale.

Five fetuses with severe hydrops were followed up serially. All showed complete resolution of both hydrops and severe anaemia after between two and five exchange transfusions in utero. In these fetuses the haemoglobin concentration increased from 34 (19) to 84 (13) g/l (p<0·005, repeated measures ANOVA). As the signs of hydrops disappeared, there was a significant decrease in concentrations of atrial natriuretic factor (p<0·02 by repeated measures ANOVA, fig 2).

Discussion

Immunoreactive atrial natriuretic factor has been found in the atria of human and rat fetuses early in gestation.[11 12] This study, in common with several others,[1 13 14] has shown that human fetuses under normal or pathological conditions have a wide range of circulating atrial natriuretic factor concentrations. Animal and human fetuses have higher concentrations of atrial natriuretic factor than their mothers.[2 5 12 14] Although atrial natriuretic factor may cross the placenta,[2] the small increases in maternal concentrations during pregnancy do not explain the pronounced increases that we found in fetuses with hydrops.[15] Most evidence suggests that the placenta is not an important site of atrial natriuretic factor degradation,[6 7 14] but arteriovenous differences in umbilical cord blood atrial natriuretic factor concentrations have been reported.[12] Kojima *et al* reported mean (SD) normal cord blood atrial natriuretic factor values of 61·3 (22·5) pg/ml,[17] which are lower than those found by Yamaji *et al*, of 165

(27) pg/ml.[13] Normal human fetuses studied by Robillard and Weiner between 22–26 weeks' gestation had mean (SEM) plasma atrial natriuretic factor values of 90 (22) pg/ml.[1] No changes in the concentration of atrial natriuretic factor with advancing gestational age have been reported.[1,14] Our study group included only one normal fetus, but the circulating atrial natriuretic factor concentrations in this and other fetuses with mild anaemia and no evidence of hydrops were well within this range, suggesting that mild decreases in fetal haemoglobin concentrations are not associated with pronounced increases in circulating atrial natriuretic factor.[18]

Hydrops fetalis commonly occurs when the fetal haemoglobin concentration drops below 60–70 g/l as in our study,[9,19] but the hydrops can be reversed with intravascular intrauterine transfusions, which raise fetal haemoglobin concentrations.[9] In Rh isoimmunisation fetal oedema results from the extensive erythropoiesis in the fetal liver that disrupts the portal circulation and impairs albumin synthesis,[9,20] and perhaps also from the cardiovascular adaptations that are also seen in anaemic fetuses.[21] Our data show that fetuses with severe hydrops had high atrial natriuretic factor concentrations and were also significantly more anaemic than those fetuses with either mild or no hydrops. In addition, an inverse relationship was found between the haemoglobin and atrial natriuretic factor concentrations.

Several factors may explain our findings. Firstly, as Rh isoimmunised fetuses become progressively more anaemic, their oxygen content is appreciably reduced. Haemoglobin concentrations of less than 40 g/l may lead to tissue hypoxia as measured by increased lactate concentration in the fetal circulation.[22] Hypoxia may then result in myocardial dysfunction and increases in umbilical venous pressure, with subsequent release of atrial natriuretic factor.[18] Although we did not measure blood gases or lactate, all our fetuses with severe hydrops and high atrial natriuretic factor concentrations had haemoglobin concentrations associated with tissue hypoxia.[22] It is likely that the tissue hypoxia in severely anaemic fetuses results more from decreased oxygen content than from lower oxygen tensions (hypoxaemia); fetuses with immune haemolytic anaemia and hydrops have similar oxygen tensions to control fetuses.[1] In sheep fetuses acute hypoxia produced by infusion of carbon dioxide to the maternal trachea is a potent stimulus for the release of atrial natriuretic factor.[23] Thus it seems that tissue hypoxia resulting from either a low oxygen carrying capacity (severe anaemia) or hypoxaemia, may induce atrial natriuretic factor release. Whether one mechanism is more potent than the other, or whether chronic rather than acute changes in tissue oxygenation are responsible for atrial natriuretic factor release in Rh isoimmunised human fetuses is unknown.

Acute volume expansion in utero also increases circulating atrial natriuretic factor concentrations in both human and sheep fetuses.[1,15] This is unlikely to explain our findings, as all samples were taken before the start of each procedure. Furthermore, hydropic fetuses are generally not hypervolaemic, but rather normovolaemic or hypovolaemic.[24–26] Arginine vasopressin and corticosteroids may also stimulate atrial natriuretic factor secretion.[2,27] Weiner recently reported lower concentrations of arginine vasopressin in anaemic fetuses requiring transfusion than in those that were not anaemic.[18] This makes atrial natriuretic factor release induced by arginine vasopressin a less likely explanation of our findings. Stimulation of atrial natriuretic factor release by sympathetic hormones is also unlikely, as human fetuses with Rh isoimmunisation have concentrations of adrenaline and noradrenaline comparable with control values.[14] There were significant reductions in plasma atrial natriuretic factor concentrations after treatment with intravascular transfusions in utero, which resulted in appreciable improvement in haemoglobin concentrations and complete resolution of the hydrops. This suggests that as oxygen delivery improves the hypoxic stimulus for atrial natriuretic factor secretion ceases. An increase in atrial natriuretic factor degradation by the kidney or other fetal organs as the fetal condition improves, however, cannot be ruled out.

In summary we have shown that human fetuses with severe degrees of anaemia and hydrops fetalis have high concentrations of atrial natriuretic factor in plasma. These high concentrations decrease as the hydrops resolves and the haemoglobin concentration increases, with treatment by intravascular transfusion in utero. The pronounced increases in the concentration of atrial natriuretic factor in fetuses with severe anaemia may be the result of a hypoxic stimulus for the release of atrial natriuretic factor.

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Antenatal diagnosis of abdominal wall defects: a missed opportunity?

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Abstract

A review of six years' experience with antenatal diagnosis of abdominal wall defects by ultrasound showed its impact to be limited by poor detection rates. Twenty infants with exomphalos and 20 with gastroschisis were recorded but only 25 (63%) were diagnosed antenatally. The ultrasound false negative rate was higher for exomphalos (35%) than for gastroschisis (22%).

No difference was detected in the incidence of associated abnormalities, premature gestation, primary closure rate, or mortality between the antenatally and postnatally diagnosed groups for either exomphalos or gastroschisis.

Antenatal diagnosis of gastroschisis has little effect on management but allows parental counselling and in utero transfer. The frequency of concomitant abnormalities in exomphalos profoundly affects prognosis and the detection of these is the major role of antenatal diagnosis in this condition.

Failure to detect abdominal wall defects by ultrasound may be a reflection of technique or equipment, but some gastroschises may be of perinatal onset and not detectable antenatally.

The advent of obstetric ultrasound has made the prenatal diagnosis of abdominal wall defects commonplace and allows rational antenatal and perinatal management. The high incidence of associated abnormalities in fetuses with exomphalos has resulted in the recommendation that full antenatal evaluation be performed in every case.[1] Although these recommendations need not apply to gastroschisis, where the incidence of associated anomalies other than gastrointestinal is extremely low, specific anatomical features may predict the postoperative course and enable more accurate antenatal counselling.

With improvements in the standards of antenatal ultrasound the opportunity exists for early diagnosis of all babies with abdominal wall defects and the detection of any associated lethal anomalies. We have undertaken this review of our recent experience with abdominal wall defects to assess the impact of antenatal diagnosis on management and outcome.

Methods

Records of all patients with antenatally diagnosed gastroschisis or exomphalos managed at this centre from January 1982 to December 1988, were entered prospectively on to a computer database. Over the same period, similar cases not antenatally diagnosed were identified from ward admission records and theatre registers. Information on prenatal ultrasound scans, place, mode and timing of delivery, associated abnormalities, operative findings, and outcome were obtained from the database, maternal and infant notes, and ultrasound records, for both the antenatally and the postnatally diagnosed groups.

Premature gestation was defined as less than 37 completed weeks of pregnancy by expected or scan dates.

It was our policy over this period to evaluate all antenatally diagnosed cases of exomphalos with karyotyping and detailed ultrasound. Echocardiography only became available in the latter part of the period.

Results

Over the period studied a total of 40 infants with abdominal wall defects (20 with gastroschisis and 20 with exomphalos) were managed at this centre, including one case of Cantrell's pentalogy and one of cloacal extrophy.

Mortality rates were similar for those diagnosed antenatally and those in whom the diagnosis was made at birth, being in the order of 45% for exomphalos and 15% for gastroschisis (table 1). Of the 12 total deaths nine had associated abnormalities (seven of the nine in exomphalos and two of the three in gastroschisis).

There was a high incidence of premature delivery in both conditions, with seven infants with exomphalos and 10 with gastroschisis delivering before 37 weeks of completed pregnancy.

A high incidence of concomitant abnormalities was found in exomphalos (12 of 20, table 2), but these all remained undetected prenatally

Table 1 Outcome

	Exomphalos		*Gastroschisis*	
	Diagnosed antenatally (n=11)	*Diagnosed at birth (n=9)*	*Diagnosed antenatally (n=14)*	*Diagnosed at birth (n=6)*
Delivery:				
Preterm	6	3	8	2
>37 weeks	4	6	6	4
Termination	1			
Surgical closure:				
Primary	7	7	11	5
Staged	2	1	3	1
No surgery	2	1		
Mortality:	5*	4	2	1
Rate	45%		15%	

*One termination.

Table 2 Associated anomalies in exomphalos

Diagnosed antenatally (n=11)	*Diagnosed at birth (n=9)*
Cleft lip	Beckwith's syndrome
Patent ductus arteriosus	Cantrell's pentalogy*
Patent urachus+duplex kidneys	Cloacal extrophy*
Trisomy 13+ileal atresia*	Multiple anomalies*
Phocomelia+cleft lip and palate	
Trisomy 18+cardiac anomalies*	
Trisomy 18 (termination)*	
Beckwith's syndrome+pulmonary hypoplasia*	

*Died.

apart from the patent urachus and a case of trisomy 18. This contrasts with an incidence of three associated abnormalities, all of which were gastrointestinal, in a total of 20 gastroschisis. Of the three cases of trisomy associated with antenatally diagnosed exomphalos, one was terminated, one was misdiagnosed as gastroschisis (see discussion), and one presented too late to consider termination.

Thirteen (52%) of those diagnosed antenatally were diagnosed at less than 20 weeks' gestation. Of the 11 infants with exomphalos, the mean gestational age was 20 weeks (range 15–35) and of the 14 with gastroschisis, the mean gestational age was 24 weeks (range 18–37). In most cases in which the diagnosis had not been made until after 30 weeks' gestation, earlier scans had not been performed. Unfortunately the indication for scanning in most of this group was unascertainable.

Two infants had normal scans at 18 and 14 weeks only to have an abdominal wall defect detected on subsequent scans later in pregnancy, one at 35 weeks (exomphalos) and one at 37 weeks (gastroschisis), respectively.

Of the 15 patients diagnosed at birth, 10 had antenatal ultrasound scans performed (table 3). The indication for scanning in these 10 was routine (for dates) (n=5), maternal age (n=1), uncertain lie (n=1), and unknown (n=3). In six a scan had been performed after 20 weeks' gestation, and in four after 30 weeks. None of these scans detected the abdominal wall defects.

Overall 10 (29%) of the abdominal wall defects (six (35%) with exomphalos and four (22%) with gastroschisis) in this series were not diagnosed antenatally despite having had scans performed.

The mode of delivery was altered in seven of 11 with exomphalos and five of 14 with gastroschisis resulting in elective termination (n=1), caesarean section (n=9), and induction (n=2). The decision to alter the mode of delivery was made by the obstetrician without reference to the paediatric surgeon.

Table 3 Undetected abdominal wall defects in infants diagnosed at birth

	Exomphalos (n=9)	*Gastroschisis (n=6)*
No antenatal scan	3	2
Negative scans	6	4
Gestational age at last scan (weeks):		
<20	2	2
20–30	1	1
>30	3	1

The false negative rate was 35% for exomphalos and 22% for gastroschisis.

The site of delivery was altered by in utero transfer in 12 of the infants diagnosed antenatally to enable the mother to be close to the baby after delivery and to avoid the risks of neonatal transfer. In eight in utero transfer was not necessary as the family lived within the catchment area of the unit. In the remainder premature delivery took place at the centre of origin before transfer could be arranged. The timing of delivery was altered in three instances by termination (n=1) and induction (n=2).

Primary surgical repair was achieved in 14 of 20 infants with exomphalos and 16 of 20 with gastroschisis. Staged closure was required in three infants with exomphalos, of whom two died (pulmonary hypoplasia and venous infarction of bowel), and in four with gastroschisis, all of whom survived.

Counselling was given by a paediatric surgeon in all but six of the 25 abdominal wall defects detected antenatally. Those not counselled included three cases of exomphalos. Failure to counsel was due to premature delivery in two, counselling by outside sources in one, and was unclear in the remainder.

Discussion

The antenatal diagnosis of abdominal wall defects by ultrasound is now widespread and usually straightforward. The criteria for ultrasound diagnosis are well reported.[2,3] Differentiation between exomphalos and gastroschisis is essential because of the association of chromosomal and lethal structural abnormalities in 30–80% of fetuses with exomphalos,[1,4] and the virtual absence of such abnormalities in fetuses with gastroschisis.[1,5] In one case in this series the anatomical features distinguishing these two conditions were not fully understood by the ultrasonologist and a mother carrying a baby with exomphalos and trisomy 13 was counselled antenatally as for gastroschisis, with disastrous results. All personal performing antenatal ultrasound scans should be fully conversant with the embryology, anatomy, and prognosis of all major structural abnormalities as recommended by the Royal Colleges of Obstetricians and Radiologists.[6] Access to specialists in the relevent postnatal field (for example, cardiologists, paediatric surgeons, etc) should be readily available to the ultrasonologist and obstetrician for accurate parental counselling.

The implications of an antenatal diagnosis of exomphalos have led to the recommendation of full evaluation of the fetus by detailed ultrasound, karyotyping, and echocardiography to enable rational decisions about the fate of the pregnancy and perinatal management.[1,7,8] In our series one pregnancy was terminated, and in a further case described above, termination would have been requested if the associated trisomy had been detected antenatally. In other reports the incidence of aborted fetuses (spontaneous or induced) is high.[4] Our rate is undoubtedly an underestimate as it takes no account of pregnancies managed without our knowledge.

Apart from a recent report[9] there is general agreement that caesarean delivery for abdominal wall defects has no beneficial effect on survival.[1 7 8 10] We do not advise caesarean section for those defects antenatally diagnosed, but there remains an increased use of this mode of delivery. This may reflect the increased complications associated with premature delivery, but may in part be due to a reduced threshold for caesarean section in these babies among obstetricians managing such pregnancies. All postnatally diagnosed babies were delivered vaginally without any apparent deleterious effects.

Antenatal ultrasound does not appear as efficient in detecting abdominal wall defects as might be anticipated. Of the 17 babies with exomphalos who were scanned, the defect was noted only in 11 (65%). This seems rather a low number but is similar to a previous report.[7] The detection rate was better for gastroschisis (78%), an improvement on the previous reported rate of 34%.[7] However, ultrasound failed to detect abdominal wall defects even well into the third trimester. This may be partly due to errors in technique, quality of equipment, and operator inexperience. Because of the rarity of abdominal wall defects, with an incidence of about 1:2550 pregnancies,[4] individual experience with these conditions will be small. Routine scanning performed for gestational age assessment is less likely to detect the defect than is detailed scanning performed for obstetric abnormalities such as a raised α fetoprotein concentration or polyhydramnios. All three of the missed exomphalos cases scanned after 30 weeks were routine scans for dates or fetal lie (two of the defects were minor). There is also the possibility that some abdominal wall defects, particularly gastroschises, may arise late in gestation or even perinatally.[5] One of our cases had a detailed anomaly scan, including fetal abdominal circumference measurement, performed by an experienced fetal ultrasonologist at 33 weeks' gestation with no abnormality detected, yet the baby was born with gastroschisis. Similar cases have been reported.[11]

Our overall mortality of 15% for gastroschisis and 45% for exomphalos is in keeping with other series,[3 4] although the mortality rate may rise as high as 86% in exomphalos if associated with cardiac defects.[4]

In our experience antenatal diagnosis does not appreciably alter the management or outcome in gastroschisis. It does allow in utero transfer of most babies for delivery at an obstetric unit with immediate access to paediatric surgery, and prenatal parental counselling. The value of counselling in this situation has yet, however, to be fully assessed. Some indication of likely outcome and severity of the postnatal course in gastroschisis, may be determined from ultrasonically detectable features such as bowel wall thickness and gut loop distention.[12]

The presence of associated anomalies with antenatally diagnosed exomphalos, profoundly affects management and outcome. In cases with associated lethal anomalies termination may be recommended, but the low mortality for isolated exomphalos[13] should be appreciated and therapeutic termination cannot be justified in this situation.

Ultrasound should detect the presence of liver or spleen in the sac; this is associated with high mortality.[7 13] Some of this excess mortality is secondary to pulmonary hypoplasia with related chest deformity[13] and chest wall diameter may prove to be a further ultrasound predictive factor of outcome in exomphalos.

Because of the low rate of detection, antenatal diagnosis has had only a limited impact on the management of abdominal wall defects. Increased awareness of abdominal wall defects and use of detailed antenatal ultrasound performed as a screening procedure in all pregnancies may improve detection. The rarity of the condition and the volume of work presenting to obstetric ultrasound departments, however, are likely to result in a substantial proportion of abdominal wall defects remaining undiagnosed during pregnancy for the foreseeable future.

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Effect of indomethacin on binding of bilirubin to albumin

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Abstract

The effect of indomethacin on the binding of bilirubin to protein was studied in vitro by the horseradish peroxidase oxidation method. Plasma indomethacin concentrations ranging from 1500 µg/l to 4500 µg/l, which were much higher than the therapeutic concentrations achieved in vivo, were used for the studies. The bilirubin-protein titration curves obtained indicate that indomethacin, although protein bound, does not affect the binding of bilirubin to protein. Our study confirms that it is safe to use conventional doses of indomethacin in jaundiced preterm infants.

Patent ductus arteriosus is common among premature infants, and indomethacin, a prostaglandin inhibitor, is widely used to induce closure. One of the alleged contraindications for its use in preterm neonates is hyperbilirubinaemia.[1 2] As the drug is mainly bound to albumin it may have the potential to displace bilirubin from its albumin binding.[3] The ability of indomethacin to induce closure of the ductus is considerably reduced when it is used after 10 to 14 days postnatal age,[4] but as hyperbilirubinaemia is a common problem among Chinese preterm infants during the first two weeks of life, we had been postponing the use of indomethacin until the hyperbilirubinaemia had subsided. We therefore had a high rate of failure to close the ductus in our preterm infants.

We report here the effects of indomethacin on the binding of bilirubin to albumin assessed in vitro by the horseradish peroxidase oxidation method.

Material and methods

The intravenous preparation of indomethacin (Merke Sharp and Dohme) was evaluated. Bilirubin–protein titration studies were carried out by the horseradish peroxidase oxidation method described by Jacobson and Wennberg.[6] Horseradish (Sigma) and pooled cord blood serum were used. Lyophilised indomethacin powder was added to 2 ml aliquots of serum to make up concentrations of 1500 µg/l, 3000 µg/l, and 4500 µg/l, which are multiples of the highest therapeutic concentration described according to the current recommended dose of 0·1 mg/kg–0·4 mg/kg.[7–9] The mixture containing indomethacin and phosphate buffer at pH 7·4 was incubated at 37°C for one hour. The incubated serum was then used to titrate with bilirubin by an automated peroxidase oxidation method.[10]

The peroxidase oxidation method is a sensitive enzymatic method using horseradish peroxidase for measuring low concentrations of free bilirubin. Free bilirubin is oxidised by hydrogen peroxide in the presence of peroxidase enzyme. Excess albumin inhibits the oxidation process by binding the free bilirubin. As the bilirubin and albumin equilibrate more rapidly compared with the rate at which unbound bilirubin is oxidised, the oxidation step limits the rate. The concentration of free bilirubin can therefore be determined from the initial rate of bilirubin oxidation measured by direct spectrophotometry of the incubated mixture after a timed exposure to peroxidase and hydrogen peroxide. The bilirubin to protein titration curve was then compared with a normal serum control containing no indomethacin. Phosphate buffers without serum but containing the same tested doses of indomethacin were also studied to examine the possible influence of indomethacin on the activity of the peroxidase oxidation enzyme.

Results

The amounts of free bilirubin generated from various bilirubin–albumin molar ratios in the

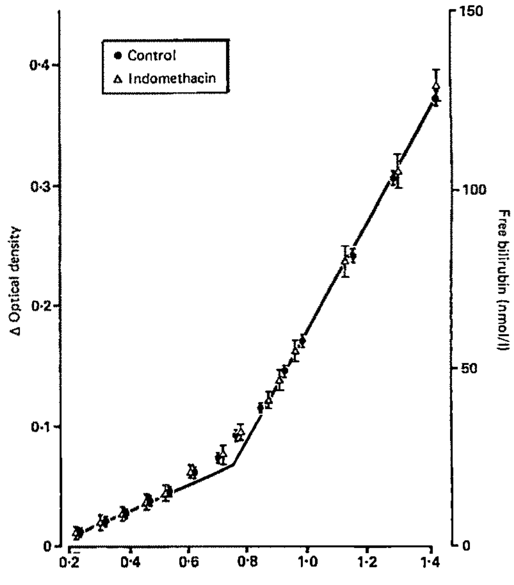

Figure 1 Titration studies gave identical results with or without indomethacin even at the highest concentration of 4500 µg/l. Titration curves at 1500 µg/l and 3000 µg/l also showed identical findings (not plotted).

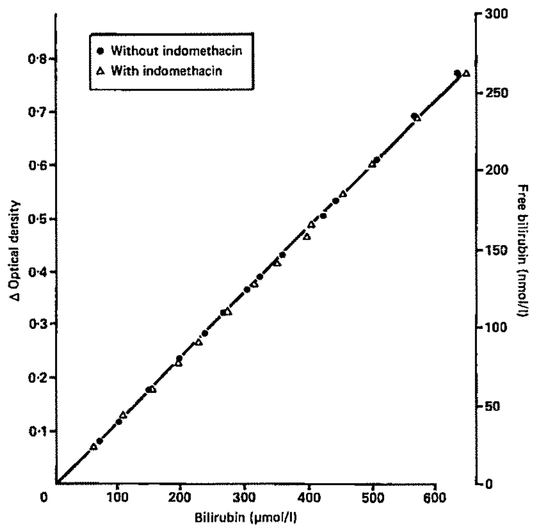

Figure 2 Linear relationship between total bilirubin in phosphate buffer solution and free bilirubin as determined by the horseradish peroxidase oxidation method. This relation is not affected by addition of high concentrations of indomethacin up to 4500 μg/l. Lower concentrations (1500 μg/l and 3000 μg/l) show identical results.

presence of 1500 μg/l, 3000 μg/l, 4500 μg/l of indomethacin were the same as that obtained from normal serum control, as shown by the bilirubin to protein titration curves (fig 1). This indicates that indomethacin, although protein bound, does not affect bilirubin–protein binding even at concentrations far higher than the therapeutic range.

There was a linear relationship (fig 2) between the amounts of total bilirubin and free bilirubin in the phosphate buffer control with and without indomethacin, indicating that indomethacin does not enhance or inhibit the peroxidase oxidation reaction.

Discussion

Indomethacin is widely used to close the patent ductus arteriosus in the neonates, but because of its protein binding properties, many workers have suggested that it should not be used in the presence of hyperbilirubinaemia. This often leads to unnecessary delay in it being given, and hence higher failure rates (as we have found among our preterm infants). Pharmacokinetic data on preterm neonates have shown that after an oral dose of 0·2 mg/kg, blood concentrations of 27–1500 μg/l are achieved, with the peak at 0·5–6 hours.[11] [12] After 0·2–0·3 mg/kg has been given intravenously, blood concentrations are between 1000 and 2500 μg/l. The half life is prolonged to as long as 40 hours in the very low birthweight infants because of immature renal and hepatic function, and also perhaps because of the enterohepatic circulation.

Mason and McQueen have found that human serum albumin possesses two classes of binding sites for indomethacin.[13] Albumin also has two binding sites for bilirubin, only one of which has a high affinity that is shared by acidic drugs.[14] It is therefore possible that bilirubin and indomethacin do not share common binding sites to albumin under usual therapeutic conditions. Some studies have shown that indomethacin in high doses may displace bilirubin.[1] [2] This may indicate that indomethacin competes with bilirubin for additional binding sites when its own are saturated.

The effect of indomethacin on bilirubin albumin binding has been studied by Shankaran *et al* using the hydroxybenzene azobenzoic acid dye binding method.[15] They used a drug concentration of 2000 μg/l and did not find any displacement of bilirubin. Recently some workers have advocated prolonging the course of indomethacin by up to a week or even 10 days to avoid reopening of the ductus.[16] Such cumulative doses of indomethacin may result in much higher blood concentrations. Furthermore, there are important individual fluctuations in indomethacin deposition making adjustment of the dose difficult even with careful monitoring. We have found no displacement of bilirubin by indomethacin even at the high concentration of 4500 μg/l. This study was, however, done in vitro at physiological pH, and one must be aware that conditions may be different in ill preterm infants who are often hypoxic, with acidaemia and electrolyte disturbances.

In summary, we found no displacement of bilirubin from albumin by indomethacin with usual therapeutic doses thus confirming that it is safe to use conventional doses of indomethacin in jaundiced preterm infants.

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A clinical trial of two parenteral nutrition solutions in neonates

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Abstract

Sixty eight neonates requiring total or supplemental parenteral nutrition in the first week of life were randomly allocated either Vamin 9 glucose (n=34) or MB233G (n=34) in a double blind trial. Twenty infants were withdrawn: four because they died before 5 days of age and 16 because the amino acids were required for less than the five days of the trial. The solutions were isocaloric (1·6 MJ/l, 380 kcal/l) and with the same nitrogen content (2·79 g/l) and were infused at rates and volumes determined by clinical staff on the basis on the infants, clinical condition and serum electrolyte biochemistry. There was an excess of deaths in the group treated with Vamin 9 glucose particularly related to babies weighing ≥1000 g. Infants <1000 g receiving the Vamin 9 glucose preparation required amino acids for twice as long. There was no significant difference between the weight losses or head circumference during the study period. Plasma aminograms in the group receiving Vamin 9 glucose showed concentrations of phenylalanine, tyrosine, proline, serine, and aspartic acid to be significantly higher than the reference range. Multiple regression analysis suggested that phenylalanine was the primary abnormality. The intravenous amino acid preparation MB233G maintained the plasma aminogram of newborn infants within the reference range of normal newborn infants.

The amino acid preparations used for intravenous nutrition of infants are designed to achieve the plasma amino acid concentrations seen after birth in the full term infant. Whether the aminogram should mimic that of the starved infant,[1] the infant fed breast milk,[2] or the infant fed on formula[3] is open for discussion.

The preterm infant in utero has a very different aminogram in the middle and last trimester to the infant once born.[4–7] It might be logical to attempt to achieve the plasma aminogram of the fetus in the preterm infant that requires intravenous nutrition if, all other things being equal, there are no signs of toxicity. We have previously reported an observational study on a group of very low birthweight infants intravenously fed with a preparation of crystalline amino acids (MB233G, Cernep Synthelabo) designed to achieve the reported cord concentrations of the infant.[5] In that study, which was not a randomised comparative study, we showed that the preparation was well tolerated metabolically with plasma aminograms in the desired range even in the smallest infants.[8]

Recently there have been reports that the more commonly used intravenous amino acid preparation in the United Kingdom, Vamin 9 glucose (KabiVitrum), is sometimes associated in very low birthweight infants with potentially toxic amino acid concentrations. It was postulated that this was an idiosyncratic effect in the babies reported.[9–12] This study reports a double blind randomised controlled trial of Vamin 9 glucose and MB233G in infants in the first week of life.

Patients and methods

From August 1986 to July 1987, 68 infants admitted to the South West Thames Regional Neonatal Intensive Care Unit at St George's Hospital, London, received intravenous amino acids during the first week of life. Prescription of parenteral support (partial or total) was by the attending clinician based on his assessment of the baby's inability to tolerate adequate nutritional support by the enteral route. These infants were randomly allocated to either Vamin 9 glucose or MB233G by pharmacy staff. Both preparations were prepared by the pharmacy in clear, orange plastic bags and giving sets as Vamin 9 glucose has a yellow colour but MB233G is colourless. A pilot study made it clear that this satisfactorily masked the nature of the preparation. The random allocation (blind to the clinical staff) was stratified to babies above and below 1000 g birth weight by the pharmacy staff. Both preparations contained the same total density of nitrogen (2·79 g/l) and energy (1·59 MJ/l, 380 kcal/l) but the differences in the amino acid constituents are shown in fig 1. The volume of infusion and electrolyte content were decided daily by the clinical staff on the basis of the clinical course and the results of the electrolyte determinations in the plasma. The amino acid (and lipid solutions) were infused continuously over 24 hours usually by peripheral infusion. Plasma amino acids and liver function tests were performed on day 1 before the start of the infusion and between 0800 and 1000 hours on day 5 if the patient was still alive and still receiving parenteral nutrition.

Small quantities of milk—either maternal expressed breast milk or preterm formula (Preaptamil, Milupa) or both—were introduced from day 1 by nasogastric tube in an attempt to promote biliary flux.

Statistical analyses were performed only for patients for whom parenteral nutrition lasted for at least five days.

Efficacy of the preparations was assessed by body weight and head circumference measure-

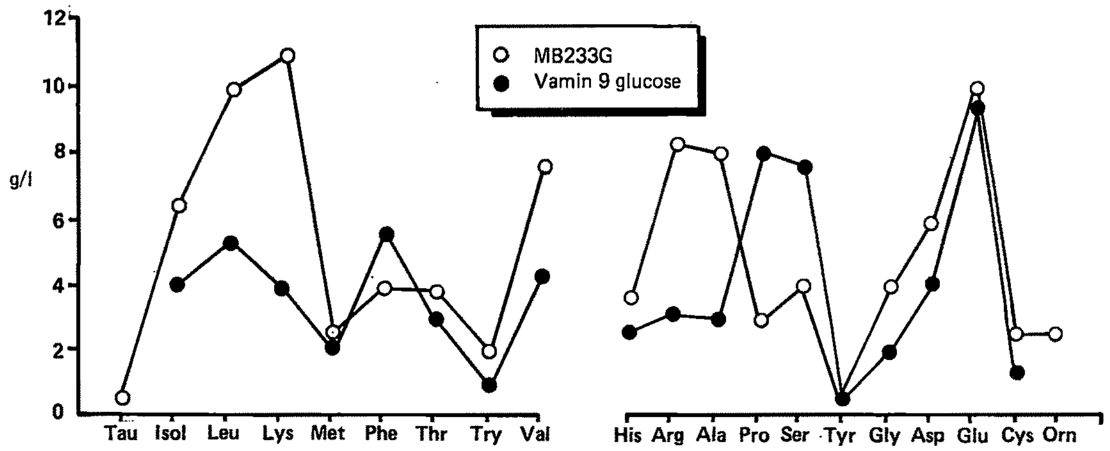

Figure 1 Amino acid concentrations in the two amino acid preparations divided according to whether they are essential (left) or non-essential (right). Tau: taurine, isol: isoleucine, leu: leucine, lys: lysine, met: methionine, phe: phenylalanine, thr: threonine, try: tryptophan, val: valine, his: histidine, arg: arginine, ala: alanine, pro: proline, ser: serine, tyr: tyrosine, gly: glycine, asp: aspartic acid, glu: glutamine, cys: cysteine, orn: ornithine.

ments and by analysis of plasma aminograms. The heparinised blood samples for the aminograms were immediately separated in a microfuge (Beckman). The plasma was deproteinised with sulphosalicylic acid (25 mg/ml plasma) and remicrofuged, the supernatant being then stored at −70°C until analysed on a Biotronik 5001 amino acid analyser.

Tolerance of the preparation was examined clinically and by frequent (at least daily) analysis of blood urea, electrolytes and haematology and acid base. Liver function tests were performed at the start and after five days of amino acid infusion.

Intravenous nitrogen, amino acid, energy, and volume intakes were compared as were the oral intakes of nitrogen, protein, energy, and volume in the two groups. Baseline data were analysed by Student's *t* test (on the ranked values) and categorical parameters by the χ^2 test or Fisher's exact test. Analysis of variance, stepwise regression analysis, and correlation were also performed on the ranked groups. Significance was defined as a p value less than 0·05. Computations and analyses were performed on SAS statistical software.

Comparisons of the amino acids in the two groups was by Wilcoxon's signed ranks test. Significance here was defined as a p value less than 0·01. When a significant difference in a particular plasma amino acid was established between the two nutritional regimes we looked for correlation between the plasma concentration and the total amino acid intake over the previous day.

The study received ethical approval from the St George's Hospital Medical ethics committee and informed consent was obtained from the parents.

Results

Intravenous nutrition was used in these infants only when they were unable to tolerate enteral feeds because of extreme immaturity or biological instability. Twenty of the 68 babies entered into the study were excluded from the analysis because the amino acid infusion was for less than five days: four babies died before the age of 5 days (one on Vamin 9 glucose, three on MB233G) and 16 babies required parenteral nutrition for less than the five day study period (eight on each preparation). This left 23 babies (n=7, <1000 g) allocated to MB233G and 25 babies (n=11, <1000 g) allocated to Vamin 9 glucose for analysis (table 1) after five completed days of intravenous nutrition.

In some of the smallest infants biochemical evaluation was less frequent than was envisaged in the study protocol for reasons of small blood volume.

COMPARABILITY OF THE GROUPS

The birthweight stratification and the weights and gestations of the babies on the two preparations were comparable and the size and maturities of the babies stratified above and below 1000 g are not appreciably different in the two nutrition groups (table 1). The clinical problems of the babies in the two groups are shown in table 2. A total of 41 (85%) of the babies were ventilated at the time of entry to the study and 27 (52%) were still ventilated on the sixth day when the study period was terminated. Despite

Table 1 Details of babies. Results are mean (SD)

	No of babies	*Gestation (days)*	*Birth weight (g)*	*Average length of parenteral nutrition (days)*	
		Weighing <1000 g			
MB233G	7	191 (13)	806 (164)	8 (2·6)	p<0·05
Vamin 9 glucose	11	187 (10)	742 (162)	13 (6·6)	
		Weighing ⩾1000 g			
MB233G	16	227 (28)	1844 (884)	14 (8·0)	
Vamin 9 glucose	14	216 (30)	1767 (939)	14 (7·5)	

Table 2 Incidence of acute clinical problems. Results are number (%)

	MB233G (n=23)	*Vamin 9 glucose (n=25)*	*p Value**
Hyaline membrane disease	15 (65)	19 (76)	0·45
Ventilated at start of end period	18 (78)	23 (92)	0·2
Ventilated at end of study period	11 (48)	16 (64)	0·35
Air leaks	2 (9)	6 (24)	0·17
Abnormal neurological signs	3 (13)	5 (20)	0·5
Appreciable sepsis	9 (39)	8 (32)	0·6
Jaundice (physiological)	10 (43)	10 (40)	0·8
Patent ductus arteriosus	6 (26)	3 (12)	0·2

*By χ^2 with Yates's correction or Fisher's exact test: all p values are non-significant.

Table 3 Nutritional intakes on first and last study day. Results are mean (SEM)

		Day 1	*	*Day 5*	*
Intravenous volume (ml/kg/day)	MB233G	101 (6)		137 (17)	
	Vamin 9 glucose	95 (8)	NS	127 (10)	NS
Intravenous amino acids (g/kg/day)	MB233G	0·29 (0·04)		1·44 (0·16)	
	Vamin 9 glucose	0·32 (0·06)	NS	1·54 (0·15)	NS
Intravenous energy (MJ/kg/day)	MB233G	0·14 (0·01)		0·21 (0·02)	
	Vamin 9 glucose	0·13 (0·01)	NS	0·18 (0·02)	NS
Intravenous nitrogen (g/kg/day)	MB233G	0·04 (0·007)		0·21 (·02)	
	Vamin 9 glucose	0·05 (0·009)	NS	0·23 (·02)	NS
Total volume (ml/kg/day)	MB233G	107 (6)		177 (13)	
	Vamin 9 glucose	104 (8)	NS	161 (6)	NS
Total oral amino acids (g/kg/day)	MB233G	0·36 (0·05)		1·87 (0·16)	
	Vamin 9 glucose	0·42 (0·06)	NS	1·93 (0·15)	NS
Total energy (MJ/kg/day)	MB233G	0·17 (0·02)		0·36 (0·02)	
	Vamin 9 glucose	0·17 (0·02)	NS	0·33 (0·02)	NS
Total nitrogen (g/kg/day)	MB233G	0·05 (0·01)		0·27 (0·02)	
	Vamin 9 glucose	0·06 (0·01)	NS	0·28 (0·02)	NS

*Column significance: non-paired *t* test.

randomisation of the intravenous nutrition, it appears that the infants in the group allocated Vamin 9 glucose may have been sicker. The respiratory problems at entry to the trial appear to be worse in the group randomly allocated Vamin 9 glucose with more babies having hyaline membrane disease and more being ventilated. Neither of these features reach significance but the fact that more infants in this group were still requiring ventilation at the end of the study and three times more had developed an air leak by the end of the study (though this trend still does not reach significance) means that the blind allocation may not have been quite as random as was hoped for. Further analysis of acid base and blood gases (submitted to the editor) showed almost identical arterial carbon dioxide tension, pH, and base excess in the two groups even when stratified into the two weight groupings. The infants <1000 g showed a trend to higher oxygen requirements and inflation pressures though again these parameters did not reach significance. Conversely, twice as many infants in the group on MB233G developed a patent ductus arteriosus; again this trend did not reach significance.

NUTRITIONAL INTAKE

The nutritional intakes are shown for the two groups in table 3. The energy, amino acid, and nitrogen provision given intravenously and the total amino acid and nitrogen intakes were similar in the two groups. The total volume and energy intakes were significantly higher on day 4 (not tabulated) in the group receiving MB233G. This was due to two infants, both less than 1000 g birth weight who had extra 5% dextrose intravenously because of large transcutaneous fluid losses. The volume and energy intakes on the other days of the study were similar. A 10% intralipid solution was introduced at 0·5 ml/kg/hour when clinical jaundice began to wane. As the trial involved only the first five days of life, in both groups this formed only a minor contribution to the total energy intake.

BIOLOGICAL EFFICACY

The efficacy of the solution was assessed clinically by the weight and head circumference changes over the time of the study (table 4). The weight losses in the two groups were similar and not unusual for the unit or babies of this gestation and size. The head circumference changes were similar in the two groups.

PLASMA AMINOGRAMS

Fourteen babies on MB233G and 16 babies on Vamin 9 glucose had plasma aminograms measured on the fifth day of parental nutrition. Eighteen (nine in each group) were not analysed due to lack of sufficient plasma (n=12) or wrong date of sampling (n=6). The individual data are shown in table 5 and the summary of the analysed aminograms is shown in fig 2. The graphic representation of the complete aminograms are given by 'stars' that show the mean

Table 4 Weight and head circumference changes

	Mean (%) weight loss (range)	*Mean (SEM) head circumferences (cm)*	
		Day 1	*Day 5*
	MB233G		
<1000 g	−4·8 (−7·3 to −1·5)	28·6 (1)	26·7 (0·9)
⩾1000 g	−0·6 (−11 to +11)		
	Vamin 9 glucose		
<1000 g	−2·5 (−10 to +13)	25·8 (1)	26·7 (0·9)
⩾1000 g	−3·5 (−19 to +5·5)		

These differences are not significant.

Table 5 Plasma amino acids at day 5 (μmol/l). Results are mean (SD)

		No of babies	*MB233G*	*No of babies*	*Vamin 9 glucose*	*Normal range*
Leucine	<1000 g	5	97·6 (47·8)	8	142·9 (67·8)	82·7–187·3
	≥1000 g	9	140·7 (65·2)	8	134·5 (50·6)	
Isoleucine	<1000 g	5	37·2 (27·6)	8	79·6 (47·6)	36·4–107·6
	≥1000 g	9	71·2 (39·3)	8	85·6 (30·6)	
Valine	<1000 g	5	175·1 (67·2)	8	244·5 (76·1)	171·8–314·2
	≥1000 g	9	240·3 (99·5)	8	272·5 (75·9)	
Methionine	<1000 g	5	30·5 (15·5)	8	46·8 (15·6)	20·4–49·7
	≥1000 g	9	39·4 (15·8)	8	54·6 (15·3)	
Cysteine	<1000 g	5	15·4 (5·8)	8	32·8 (18·8)	32·4–57·6
	≥1000 g	9	34·5 (8·4)	8	49·6 (24·2)	
Taurine	<1000 g	5	90·8 (34·7)	8	110·0 (96·4)	115·1–378·9
	≥1000 g	9	142·6 (69·4)	8	194·6 (115·1)	
Phenylalanine	<1000 g	5	86·4 (15·8)	8	206·1 (73·0)	48·9–99·1
	≥1000 g	9	89·4 (33·0)	8	198·5 (68·7)	
Tyrosine	<1000 g	5	91·4 (100·0)	8	368·7 (309·7)	35·8–90·2
	≥1000 g	9	61·3 (51·6)	8	386·1 (372·7)	
Lysine	<1000 g	5	195·9 (93·5)	8	198·2 (88·7)	216·4–467·6
	≥1000 g	9	322·1 (151·7)	8	201·1 (84·4)	
Threonine	<1000 g	5	144·2 (40·2)	8	281·1 (125·6)	161·3–374·7
	≥1000 g	9	182·7 (74·1)	8	299·1 (133·8)	
Histidine	<1000 g	5	88·8 (26·8)	8	121·1 (44·1)	63·4–180·6
	≥1000 g	9	119·0 (45·7)	8	117·8 (29·2)	
Arginine	<1000 g	5	46·7 (33·8)	8	54·3 (44·5)	47·5–114·5
	≥1000 g	9	90·3 (52·3)	8	49·0 (23·5)	
Aspartic acid	<1000 g	5	16·8 (10·4)	8	61·5 (70·3)	6·4–35·7
	≥1000 g	9	40·1 (50·9)	8	51·7 (32·9)	
Serine	<1000 g	5	131·9 (36·0)	8	315·1 (167·7)	143·4–214·6
	≥1000 g	9	197·0 (66·2)	8	344·1 (183·1)	
Asparagine	<1000 g	5	22·8 (13·4)	8	24·4 (12·3)	28·4–53·6
	≥1000 g	9	31·3 (18·9)	8	28·9 (25·7)	
Glutamic acid	<1000 g	5	55·2 (15·4)	8	204·7 (156·5)	58·3–313·7
	≥1000 g	9	110·0 (130·6)	8	192·4 (150·5)	
Glutamine	<1000 g	5	328·1 (111·2)	8	321·3 (131·4)	219·3–834·7
	≥1000 g	9	710·3 (680·5)	8	432·5 (130·6)	
Proline	<1000 g	5	126·6 (88·2)	8	411·5 (169·4)	10·7–252·4
	≥1000 g	9	200·9 (72·2)	8	478·3 (211·1)	
Glycine	<1000 g	5	216·8 (54·1)	8	358·6 (101·8)	199·9–338·1
	≥1000 g	9	335·7 (108·8)	8	417·2 (177·6)	
Alanine	<1000 g	5	224·8 (114·8)	8	285·5 (136·8)	281·0–595·0
	≥1000 g	9	306·3 (176·0)	8	379·7 (228·0)	
Citrulline	<1000 g	5	7·2 (3·6)	8	14·6 (4·7)	2·0–55·0
	≥1000 g	9	8·8 (4·2)	8	15·0 (11·1)	
Ornithine	<1000 g	5	80·0 (49·4)	8	78·4 (54·3)	45·4–162·6
	≥1000 g	9	137·3 (71·6)	8	98·0 (75·0)	

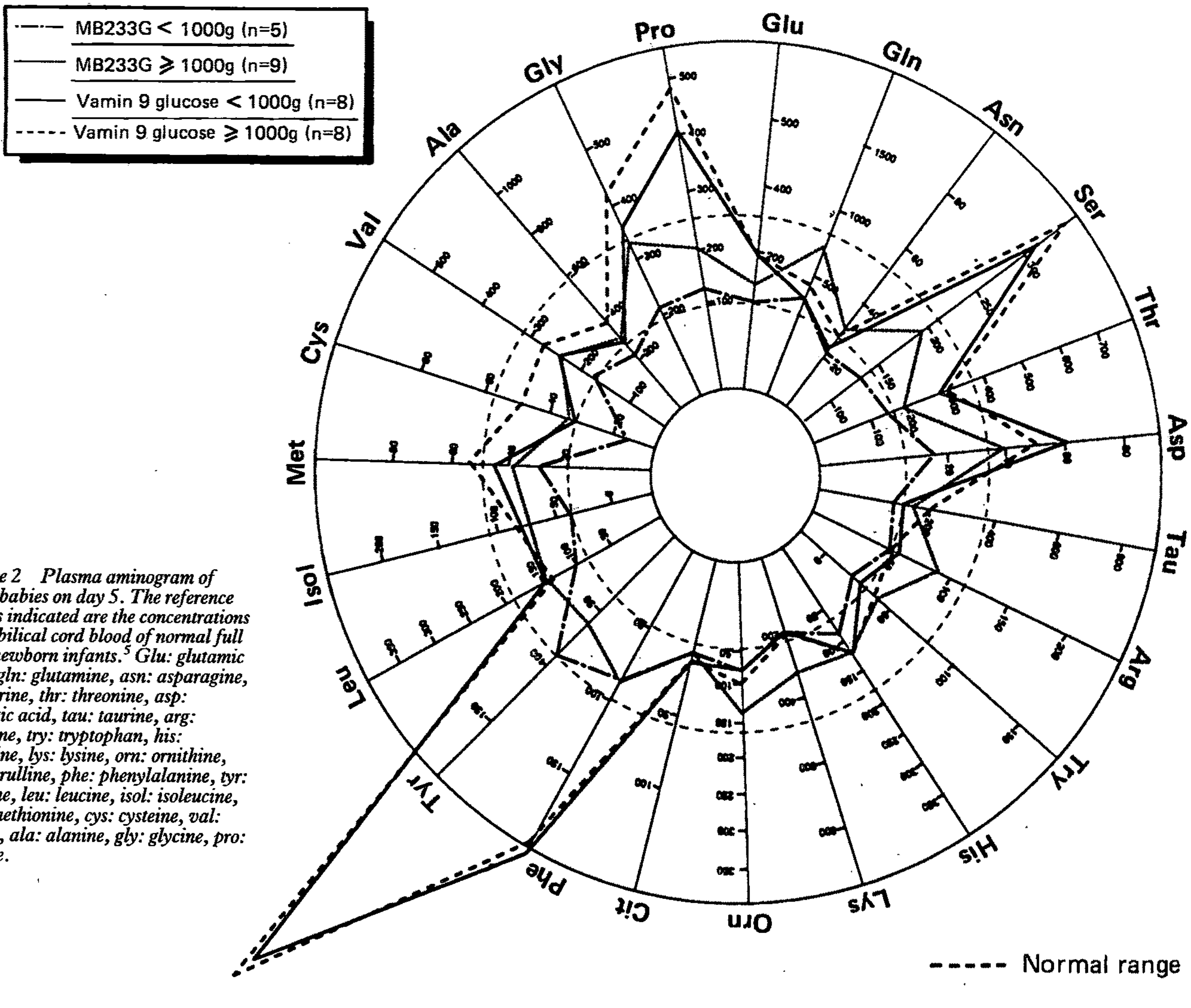

Figure 2 Plasma aminogram of study babies on day 5. The reference ranges indicated are the concentrations in umbilical cord blood of normal full term newborn infants.[5] Glu: glutamic acid, gln: glutamine, asn: asparagine, ser: serine, thr: threonine, asp: aspartic acid, tau: taurine, arg: arginine, try: tryptophan, his: histidine, lys: lysine, orn: ornithine, cit: citrulline, phe: phenylalanine, tyr: tyrosine, leu: leucine, isol: isoleucine, met: methionine, cys: cysteine, val: valine, ala: alanine, gly: glycine, pro: proline.

value of each amino acid. The dotted lines represent the reference ranges from cord blood of preterm and term deliveries.[5] The ranges are seen in table 5.

The mean values of the branched chain amino acids and the sulphur containing amino acids were similar in the two groups, although some plasma taurine concentrations in the group on Vamin 9 glucose were extremely low (for example—9, 20, 27 μmol/l; lower limit of reference range=115 μmol/l) and the mean value of the cysteine in the group on MB233G was lower than the reference range for babies less than 1000 g. The mean plasma concentrations of phenylalanine and tyrosine were significantly greater ($p<0{\cdot}0001$ and $p<0{\cdot}0003$ respectively) in the group on Vamin 9 glucose. The concentrations in the group Vamin 9 glucose were not related to the body weight of the infant. The mean plasma concentrations of lysine were slightly lower than the lower reference range value (216 μmol/l) in both groups on Vamin 9 glucose (<1000 g=198 μmol/l, ≥1000 g=201 μmol/l) and in the babies <1000 g fed MB233G (196 μmol/l). The mean plasma threonine concentrations were in the normal range for all except the group on MB233G weighing <1000 g. The difference between the two groups weighing ≥1000 g, however, reached significance ($p<0{\cdot}004$). The histidine concentrations were similar and within the normal range for all groups.

The non-essential amino acids proline and serine were higher in the group on Vamin 9 glucose ($p<0{\cdot}0001$ and $p<0{\cdot}003$ respectively). The aspartic acid concentrations were similar. Citrulline was significantly higher in the group on Vamin 9 glucose ($p<0{\cdot}009$) despite being in the normal range. The other non-essential amino acids were in the reference range and similar in the two groups.

Stepwise discriminant analysis was performed on the aminograms of the two treatment groups on day 5, using threonine, serine, glutamate, proline, citrulline, methionine, tyrosine, and phenylalanine as the exploratory variables. Phenylalanine explained on its own some 55% of the total variation between the two treatment groups but as the variability of these parameters was large, the same procedure was applied with ranked data. In this case proline, tyrosine, and serine accounted for 74% of the total variation and phenylalanine was no longer significant. These results reflect the intricacies of these variables which cannot be clarified further because of the relatively small sample size. The data confirms only that these four amino acids are most involved in the final differences between the two treatment groups.

In fig 3A we have plotted the mean intakes of the four amino acids phenylalanine, proline, serine, and tyrosine in the 24 hours before the plasma analysis on day 5 (fig 3B). The higher plasma concentrations seem related to the higher intakes in the case of the first three amino acids. With tyrosine, however, there is no apparent association of plasma concentration to the previous day's intake.

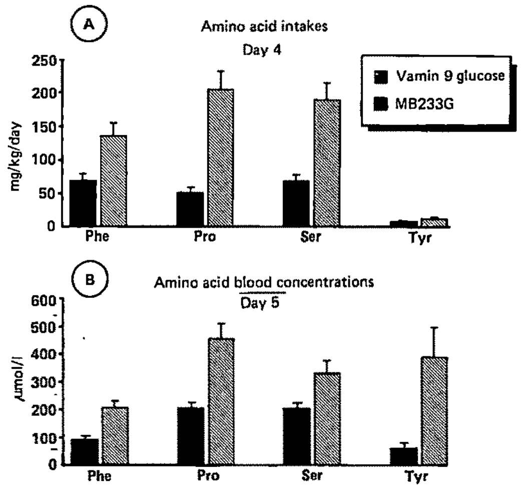

Figure 3(A) Intakes of four amino acids on day 4 and (B) amino acid concentrations on day 5. Phe: phenylalanine, pro: proline, ser: serine, tyr: tyrosine.

TOLERANCE

A total of 18 (78%) of the infants on MB233G and 23 (92%) on Vamin 9 glucose were ventilated on entry to the study, and at the end of the study period 11 (48%) on MB233G and 16 (64%) on Vamin 9 glucose remained ventilated (difference not significant by χ^2 test with Yates's correction). The oxygen requirements over the five days were similar in both groups.

Babies weighing <1000 g fed on MB233G required parenteral nutrition for significantly less time (mean (SD) 8 (4·8) days) than babies fed Vamin 9 glucose (13 (10 days), $p<0{\cdot}05$) (table 1).

Forty seven infusions were required in the 23 babies on MB233G and 54 in the 25 babies on the Vamin 9 glucose with 21 instances of drip infiltration in the group on MB233G and 29 in the group on Vamin 9 glucose.

Significantly higher blood glucose concentrations were seen on days 4 and 5 in the group on MB233G but these were related to two infants weighing less than 1000 g who received extra 5% dextrose to compensate for transcutaneous water losses.

The plasma sodium, potassium, calcium, phosphorus, and blood urea concentrations were all within the reference ranges and similar in both groups throughout the study.

The mean (SD) alanine aminotransferase, aspartate aminotransferase, and γ glutamyltransferase activities of the two groups were similar on day 1 (alanine aminotransferase: Vamin 9 glucose 4·9 (2·9) U/l, MB233G 6·9 (6·1) U/l, $p=0{\cdot}2$; aspartate aminotransferase: Vamin 9 glucose 47·5 (30·6) U/l, MB233G 30·8 (23·8) U/l, $p=0{\cdot}1$; and γ glutamyltransferase: Vamin 9 glucose 62 (33) U/l, MB233G 79 (49) U/l, $p=0{\cdot}25$). On day 5, however, these liver enzymes were significantly higher in babies fed on Vamin 9 glucose (alanine aminotransferase: Vamin 9 glucose 11·3 (9·6) U/l, MB233G 3·7 (1·1) U/l, $p<0{\cdot}05$; aspartate aminotransferase: Vamin 9 glucose 33·0 (18·4) U/l, MB233G 16·4 (10·3) U/l, $p<0{\cdot}05$; and γ glutamyltransferase 60 (28) U/l, MB233G 63 (66) U/l, $p=0{\cdot}9$).

Both groups of infants were acidotic in conventional terms throughout the study period

Table 6 Mortality

	MB233G		*Vamin 9 glucose*		*Significance by Fisher's exact test*
	Total No	*No (%) who died*	*Total No*	*No (%) who died*	
		(a) By allocation to the study			
<1000 g	11	3 (27)	16	5 (31)	NS
≥1000 g	23	0	18	4 (22)	NS
Total	34	3 (9)	34	9 (26)	NS
		(b) By completion of study protocol			
<1000 g	7	0	11	4 (36)	NS
≥1000 g	16	0	14	4 (29)	p<0·05
Total	23	0	25	8 (32)	p<0·01

but with values that were within the neonatal unit's reference range for babies with severe respiratory problems on ventilators. There was no significant difference between the two groups or apparent trend at a non-significant level (data submitted to editor and referees).

The haemoglobin concentration, white cell count, and platelets were as would be expected from the condition of these infants and were similar in the two groups.

DEATHS

Table 6 shows the mortality in the study, 6a by allocation of treatment and 6b by completion of the study protocol. Four infants died before the study protocol was completed (three on MB233G and one on Vamin 9 glucose). These infants were all <1000 birth weight and all had respiratory problems related to their extreme prematurity. Two of the infants on MB233G and the single infant on Vamin 9 glucose had some evidence of infection though only one death (an extremely growth retarded infant, 480 g birth weight and 27 weeks' gestation) was thought to be directly related to pseudomonas septicaemia and pulmonary haemorrhage from coincident disseminated intravascular coagulation.

The deaths of the infants completing the study protocol were confined to the group on Vamin 9 glucose. Four infants <1000 g birth weight died: one at 6 days (respiratory failure, septicaemia, and intra-abdominal haemorrhage), the second at 10 days (respiratory failure due to respiratory distress syndrome and intraventricular haemorrhage), the third at 17 days (respiratory failure due to respiratory distress syndrome with pulmonary interstitial emphysema and pneumothorax going on to bronchopulmonary dysplastic changes seen on radiography), and the fourth at 28 days (respiratory failure from chronic lung disease).

The other infants (1000, 1325, 2500, 3600 g birth weight) died at 10, 20, 28, and 11 days respectively. All had respiratory distress syndrome and three had air leak during the early days of life. The two smaller infants of the four had intraventricular haemorrhages of grade 3 severity and patent ductus arteriosus and eventually died of infections. The larger two infants developed severe changes of bronchopulmonary dysplasia that were seen on radiography and died of respiratory failure.

The plasma amino acid concentrations of the infants in the group on Vamin 9 glucose that died were not significantly different on day 5 (the day of study completion) from the infants fed Vamin 9 glucose who went on to survive. The mean (SD) results were as follows—phenylalanine: died 218 (82) μmol/l, alive 192 (62) μmol/l; tyrosine: died 452 (360) μmol/l, alive 312 (284) μmol/l; proline: died 476 (171) μmol/l, alive 426 (204) μmol/l; serine: died 314 (184) μmol/l, alive 327 (184) μmol/l; aspartic acid: died 69 (70) μmol/l, alive 49 (38) μmol/l. We unfortunately do not have measurements of either the plasma aminograms at a later stage, or the plasma ammonia concentrations as this was not part of the study protocol and we became aware of this apparent cluster of deaths after the trial only when the randomisation code was broken at the end of the study.

Discussion

The smaller and more immature the infant, the more crucial it is to provide adequate nutrition at a very early stage. It is commonplace now for very low birthweight infants to be given total or supplemental parenteral nutrition where any delay is expected in establishing enteral feeds.[13] Most currently used amino acid preparations have not been prepared with the (premature) newborn in mind and have been accepted as satisfactory when the aminogram in the patient receiving the preparation has fallen in the reference range of full term and well infants either starved,[1] fed on breast milk,[2] or formula.[3] It is possible that the nitrogen requirements of very immature infants will be more critical than in bigger and more mature infants because of greater metabolic immaturity and the absence of the safety valve of the placenta. Amino acid solutions with the wrong composition could produce potentially hazardous disturbances of blood chemistry such as a rise in the plasma concentrations of neurotoxic or hepatotoxic amino acids, metabolic acidosis, and hyperammonaemia.

The fetus in utero is not fed orally but is fed parenterally across the placenta. It is therefore reasonable to assume that the aminogram of the fetus in utero in the last trimester of pregnancy is unlikely to be toxic to the developing fetus and is probably satisfactory for the preterm or term newborn infant.

The aminograms of the mid trimester fetus have now been defined[6 7] and are not significantly different from the cord blood amino acids of preterm and full term normal deliveries.[4 5] The preparation MB233G is a new crystalline amino acid preparation that has been designed by Rigo *et al* to achieve plasma amino acid concentrations in these reference ranges.[14] A previous observational study in our unit looked at the tolerance and efficacy of MB233G in 42 very low birthweight infants, 19 of whom were <1000 g birth weight. We found no abnormal effects that we felt could be related to the preparation but in that study we were not comparing MB233G with another preparation.[8] Vamin 9 glucose is a well tried L amino acid preparation used in the United Kingdom and Europe and more than 70% of the isomers are retained when infused[15]; it contains no taurine or ornithine however. The absence of taurine may be important as taurine deficient diets (casein based parenteral nutrition solutions) in primates[16] and humans[17] lead to retinal degeneration and abnormal electroretinograms. The absence of ornithine in the diet is not associated with any specific dysfunction but it is recognised as an essential amino acid. Both taurine and ornithine are present in MB233G. The only other qualitative difference in the two preparations is the cysteine which in Vamin 9 glucose is cystine whereas in MB233G it is cysteine hydrochloride. Whereas in the older infant and adult cysteine is a non-essential amino acid, it is thought to be essential in the preterm infant because the absence of cystathionase in the fetal and preterm infant's liver makes the usual conversion of methionine to cysteine unlikely.[18]

The randomisation and stratification of the groups shows them comparatively well matched for the size and maturity (table 1); however, there is an unfortunate trend towards the sicker infants with respiratory problems being allocated Vamin 9 glucose. This group (table 2) was more likely, both at study entry and completion, to require ventilation and three times as many infants in the group developed air leaks. None of these features reached significance but the differences in outcome in mortality (table 6) may be related to these features.

The nutritional intakes both parenterally and enterally were very similar in the two groups both at entry to the trial on day 1 and when the trial was concluded on the fifth day (table 3). Both preparations seemed clinically well tolerated during the five days of the study but infants <1000 g fed Vamin 9 glucose required parenteral nutrition for a significantly longer period (table 1); this may be related to the babies in this group being sicker. Significantly more infants died in the period after study in the group receiving Vamin 9 glucose ($p<0{\cdot}01$, table 6); this may also be related to the infants being sicker. Against this is the fact that the difference is largely accounted for by the excess of deaths in the group weighing ≥1000 g fed on Vamin 9 glucose (p 0·05) which are a group of infants more likely to be salvageable. We hypothesise that the deaths in the group on Vamin 9 glucose may be related to the high plasma concentrations of the aromatic amino acids phenylalanine and tyrosine, which are known to be neurotoxic with long term neurological sequelae.[19 21] The concentrations of phenylalanine in the group of infants on Vamin 9 glucose in this study (mean 187 µmol/l, range 44–345) and the concentrations of tyrosine (mean 367 µmol/l, range 46–925) are up to five times higher than the upper limit of normal infants. The fetuses of phenylketonuric mothers (who can have phenylalanine concentrations in pregnancy of 600–900 µmol/l) are usually mentally retarded and microcephalic,[22] and the preterm infants of this study are going through a similar phase of extremely rapid brain growth. Abnormal concentrations of phenylalanine have been reported before but were thought to be related to extreme immaturity.[9–12] The concentrations in the babies on Vamin 9 glucose were similar in infants stratified above and below 1000 g, suggesting that immaturity might not be the sole problem. The plasma concentrations of tyrosine were also extremely high in the group on Vamin 9 glucose compared with the reference range (36–90 µmol/l) and were often at concentrations seen in transient tyrosinaemia.[20] Intellectual deficits were shown in term neonates with this condition by Menkes *et al* in 1966[19] and by Mamunes *et al* in 1976,[20] but Martin *et al* could not demonstrate abnormality in six infants followed up to six years.[23] The severe mental retardation associated with the autosomal recessive form of tyrosinaemia known as the Richner-Hanhart syndrome is preventable by the use of low tyrosine diets and the plasma tyrosine concentrations in untreated patients with this condition are similar to the concentrations seen in the infants on Vamin 9 glucose. The data in figure 3 suggest that the phenylalanine blood concentrations may be related to the amino acid intake but the high tyrosine concentrations seen in the group on Vamin 9 glucose, which were over four times those of the group on MB233G, occurred even though the intake of tyrosine in the 24 hours before sampling was the same. It appears that the tyrosine is not utilised in the group on Vamin 9 glucose and we hypothesise that this may be because of the high plasma concentrations of phenylalanine, proline, serine, or aspartic acid interfere with effective liver function. The discriminant analysis of the raw data suggests that the phenylalanine concentrations may be the primary cause of this problem.

Against the hypothesis that the high plasma amino acid concentrations were in some way associated with the deaths is the apparent lack of clinical toxicity of the preparation Vamin 9 glucose and the fact that the amino acid concentrations of the survivors were no different on day 5 in that group whether they went on to survive or die. It is noteworthy that some liver cell function tests were significantly higher on day 5 of the infusion in this group. Because the trial finished after five days of infusion we have no further measurements of plasma amino acids or of ammonia concentrations but many of the infants in both groups continued with their intravenous nutrition after their formal study period. The deaths when assessed clinically do not fit into any uniquely similar pattern but display the characteristics of very sick preterm infants requiring intensive care.

Both groups in this study had identical and adequate ascorbic acid intakes (250 mg/kg/day) so that restricted availability of the cofactor for tyrosine transferase activity is unlikely to account for the accumulation of tyrosine or phenylalanine in the group on Vamin 9 glucose.

The additional taurine in the MB233G did not lead to higher plasma taurine concentrations in this group and the mean taurine concentrations in both groups of babies weighing less than 1000 g were lower than the lower limit of the reference range. In the group on Vamin 9 glucose some infants had extremely low plasma concentrations. It is possible that the taurine content in MB233G prevented such low concentrations.

We conclude from this study that preparation MB233G maintains the plasma aminogram more closely within the normal reference range related to cord blood amino acid concentrations than does Vamin 9 glucose where the plasma phenylalanine, tyrosine, proline, serine, and aspartic acid are high independent of the weight of the infant.

Although during the study both preparations seemed well tolerated, the excess mortality in the group on Vamin 9 glucose after the end of the study period must be noted and although it may be related to sicker infants being present in this group, the association is independent of birth weight and is coincident with high plasma amino acid concentrations of some known neurotoxic and hepatotoxic amino acids. This must remain a cause of concern until the study can be repeated by others.

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Reproducibility of cerebral artery Doppler measurements

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Abstract

Intraobserver and interobserver variability were calculated for the measurement of cerebral artery pulsatility indices by five observers who made two recordings on each of 12 stable preterm infants. Variations in pulsatility indices of up to 0·21 were found; no measurement was below 0·55.

There have been many studies of the cerebral circulation of newborn infants with Doppler techniques since the report by Bada *et al* in 1979, in which they used the Pourcelot or pulsatility index.[1] Pulsatility index is defined as (S–D)/S, where S is systolic and D is diastolic flow velocity.[1] It has been suggested that a low pulsatility index (<0·55) during the first five days of life is a useful prognostic sign, with a high sensitivity for the prediction of death or disability, in infants who have been subjected to severe birth asphyxia.[2] The aim of this study was to assess the reproducibility of measurement of pulsatility indices by clinical staff using a commercial duplex scanner.

Patients and methods

Twelve stable preterm infants were each studied within a period of one hour by five observers, using a duplex Doppler scanner (Ultramark 4, Advanced Technical Laboratories). A real time image was obtained in the parasagittal plane, and a Doppler signal obtained at 5 MHz from the anterior cerebral artery anterior to the corpus callosum. Each observer made two separate recordings from the anterior cerebral artery of each infant, and stored the flow velocity waveforms on a hard copy. The hard copies were kept by one of the authors who, after collection of the data was complete, issued each observer with the 24 recordings he had made from the 12 infants. Observers were therefore unaware which were paired results from the same infant. Systolic and diastolic velocities were measured by ruler on the hard copy. Measurements were made to the nearest millimetre and the pulsatility index was calculated as the mean (S–D)/S of at least three waveforms.

Analysis of variance was used to study the 60 first observations by each observer on each infant.[3] Estimates of the SD of both the intra-observer and interobserver variability were calculated, making the reasonable assumption of no interaction between baby and observer.

Median and range of values for pulsatility indices of 12 infants

Case No	*Pulsatility indices*	
	Median	*Range of five values*
1	0·81	0·77–0·85
2	0·82	0·72–0·93
3	0·71	0·63–0·78
4	0·76	0·73–0·83
5	0·75	0·70–0·83
6	0·78	0·75–0·85
7	0·74	0·69–0·80
8	0·73	0·60–0·80
9	0·79	0·75–0·81
10	0·81	0·75–0·88
11	0·75	0·69–0·76
12	0·78	0·73–0·81

Results

The table shows the medians and ranges of values obtained by the five observers from each of the 12 infants. There was considerable variation between observers, the most extreme being between 0·72 and 0·93. None of the observers recorded a pulsatility index below the 0·55 threshold suggested by Archer *et al.*[2] The estimates of the SDs associated with the intra-observer and interobserver variances were 0·046 and 0·030, respectively.

These values may be interpreted as follows: (1) Of the differences between two independent measurements made by the same observer on the same patient, 95% will be less than 0·13 $(=2{\cdot}83\times0{\cdot}046)$ or 17% when expressed as a percentage of the mean pulsatility index.[4] (2) Of the differences between two independent measurements made by two observers on the same patient, 95% will be less than 0·16 $(=2{\cdot}83\times\sqrt{0{\cdot}046^2+0{\cdot}030^2})$, or 20% as a percentage of the mean pulsatility index.

Analysis of the differences between the duplicate determinations by each observer showed that these differences were less than would be predicted if each was subjected independently to the calculated degree of intra-observer variability. The two measurements were therefore not truly independent, and the duplicate measurements were not used in the analysis.

Discussion

Previous studies of the reproducibility of measurement of pulsatility indices have been limited to comparison of results obtained by two or three experienced observers.[2 5 6] As cerebral artery Doppler studies are now useful clinically as well as in research, we were interested to find out how reproducible the technique was when used in routine clinical practice. We therefore used a commercially available duplex scanner, and measurements were made by the five people who were responsible for the routine ultrasound surveillance of high risk newborn infants on our nursery. Two of these observers were research personnel and three were in clinical service posts; three had less than one year's experience in ultrasound and Doppler techniques.

If the mean pulsatility index for all observers is taken as the most accurate estimate of the 'true' pulsatility index, then this small sample did not contain any infant with a pulsatility index below 0·55. It was therefore not possible to determine the risk of a true low pulsatility index being missed by an individual observer. There was no false positive measurement of a pulsatility index less than 0·55. Incomplete insonnation of a vessel can give the false impression of low or absent diastolic flow and therefore a high pulsatility index, whereas the finding of high diastolic flow and low pulsatility index is much less likely to be the result of observer error. There is therefore less chance that a clinically important error would be made in neonatal cerebral artery Doppler studies than in obstetric scanning, where absent or reduced diastolic flow is the main diagnostic feature.

This study showed that interobserver variability did not exceed intraobserver variability in the measurement of pulsatility index using a duplex Doppler scanner. In some infants, however, measurements of pulsatility index varied widely, and it would therefore seem sensible not to make clinical judgments on a single pulsatility index estimate. This small study was confined to preterm infants, none of whom had a pulsatility index in the pathological range. Reproducibility of measurement of pulsatility indices over a wider range of normal and abnormal values should be established if the pulsatility index is to be used as a prognostic indicator.

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Virilisation of female preterm infants

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Abstract

Two cases of hypertrophy of the clitoris in premature girls are reported; this was associated with persistently high concentrations of adrenal fetal zone androgens.

Isolated hypertrophy of the clitoris in neonates is known to be associated with congenital adrenal hyperplasia, administration of androgens to the mother in the second and third trimesters of pregnancy,[1] and maternal androgen secreting tumours. We report two cases of progressive hypertrophy of the clitoris developing after birth, and associated with persistent excretion of high concentrations of 3β-OH-5-ene steroids, produced by the fetal adrenal zone and which are known to be weak androgens. This association has not previously been reported.

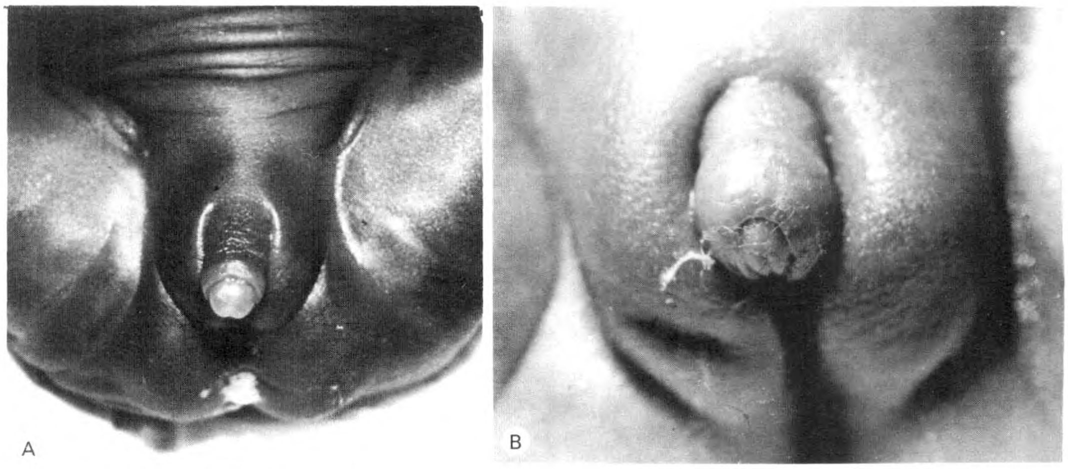
Case reports

CASE 1

A girl, birth weight 640 g, was one of twins delivered at 24 weeks' gestation for antepartum haemorrhage; the mother was aged 24, para 1+1. The second twin died aged 11 hours.

The surviving twin developed hyaline membrane disease, and was ventilated for five weeks, remaining oxygen dependent for a further seven weeks. Apnoea of prematurity was treated with theophylline from 4 to 10 weeks. She had a patent ductus arteriosus, which failed to close with indomethacin, and led to treatment with digoxin and frusemide from 9 weeks until spontaneous closure at 11 weeks. She had recurrent hyponatraemia and required sodium supplements.

Clitoromegaly was noted at 4 weeks of age (fig 1A). She had a left inguinal hernia with a palpable gonad (a macroscopically normal ovary was later found at herniotomy). Ultrasound showed a normal uterus and adrenals. The karyotype was normal female. Plasma 17α-OH progesterone was 27·2 nmol/l at 7 weeks, 61·8 nmol/l at 9 weeks, and 11·2 nmol/l at 20 weeks. Adrenocorticotrophic hormone (ACTH) was 4·8 pmol/l at 11 weeks and 6·6 pmol/l at 20 weeks (immunoradiometric assay, Eurodiagnostics, Netherlands). The plasma cortisol on five occasions, when not receiving hydrocortisone, ranged from <69 nmol/l to 316 nmol/l and after corticotrophin stimulation increased to >1380 nmol/l at 60 minutes. Plasma dehydroepian-



Clitoromegaly in case 1(A) and case 2(B).

drosterone sulphate (DHAS) was 6·8 μmol/l at 12 weeks and 2·5 μmol/l at 20 weeks. At 12 weeks plasma testosterone was 0·5 nmol/l. The results of serial 24 hour urinary steroid analysis using gas chromatography with capillary column[2] are shown in the table.

Hydrocortisone 1·6 mg/kg/day was given from 13 weeks to 19 weeks on the advice of an endocrinologist because it was felt that congenital adrenal hyperplasia had not been ruled out at the time. At the age of 3 years, when she was admitted for surgical reduction of the clitoris, a 24 hour urinary steroid profile was normal, as were plasma DHAS and 17α-OH progesterone concentrations.

CASE 2

A girl, birth weight 1056 g, was born to a mother aged 36 years, para 2+3, who had had one neonatal death at 26 weeks. The pregnancy was complicated by vaginal blood loss and cervical dilation at 19 weeks. A cervical suture was inserted and labour suppressed with ritodrine and indomethacin. Maternal serology was positive for toxoplasmosis and she was treated with spiramycin. Premature rupture of membranes occurred at 22 weeks and there was spontaneous vaginal delivery at 27 weeks' gestation.

The baby had mild respiratory distress requiring oxygen for three days, and was given benzylpenicillin and gentamicin; theophylline was given for apnoea. She was tube fed with expressed breast milk containing disodium hydrogen phosphate supplements. Additional sodium chloride was given for hyponatraemia. There was no clinical or serological evidence of congenital toxoplasmosis.

A large clitoris was noted at 6 weeks of age (fig 1B). Blood pressure was normal. Chromosomes showed a normal female karyotype. Plasma 17α-OH progesterone at 8 weeks was 22 nmol/l. The 24 hour urine steroid excretion rates are shown in the table. Plasma ACTH was 10·3 pmol/l at 8 weeks and 10·1 pmol/l at 10 weeks. Cortisol concentrations ranged from <60 to 137 nmol/l.

She was discharged home at 8 weeks. Clitoromegaly gradually resolved over the next three months.

24 hour urinary steroid excretion rates. The results from two full term infants and from two female preterm infants who were not virilised are given for comparison

	Postnatal age (weeks)	*Total cortisol metabolites (μg/kg/day)*	*3β-OH-5-ene metabolites (μg/kg/day)*
Case 1	12	<10	1730
	20	40	500
Case 2	9	220	1410
Preterm 1	13		924
Preterm 2	12		1172
Term (n=2)	8	100	130

Discussion

Virilisation of female newborn infants is usually caused by excess androgen production due to an inborn error of steroid metabolism. This is not thought to be the cause in these two infants because the virilisation was not present at birth and urine steroid profiles showed no evidence of 21 or 11β hydroxylase deficiency. Furthermore, plasma 17α-OH progesterone concentrations were not as high as those reported in congenital adrenal hyperplasia and were within the range reported for preterm infants without congenital adrenal hyperplasia.[3 4]

The plasma and urine measurements (table) show that both of these cases continued to excrete 3β-OH-5-ene steroids, which are weak androgens, long after the virilisation was first noticed, at concentrations which in term babies would only be found in the first few days of life.[5] The persistence of the 3β-OH-5-ene steroids (mainly dehydroepiandrosterone (DHA) and its metabolites) in preterm infants has been described,[4] but the association with virilisation of female infants has not. The cause of the persistent secretion of these fetal zone steriods has never been satisfactorily explained. There is evidence that ACTH regulates the adrenal cortex before and after birth, though other trophic factors may also be involved.[6] In cell culture ACTH will stimulate fetal zone cells to produce DHA (and cortisol). In utero this DHA would be exported to the placenta and aromatised to oestrogens, a route of disposal not available to the infant after birth. The persistent 3β-OH-5-ene steroid excretion might be caused by excess ACTH drive, due for example, to inadequate cortisol synthesis secondary to diminished activity of 3β hydroxysteroid dehydrogenase. The concentrations of ACTH and of cortisol observed in the plasma, however, do not support this hypothesis. The persistent rise of the 3β-OH-5-ene steroids might therefore be due to the response of an adrenal cortex, consisting predominantly of DHA secreting fetal zone cells, to the normal ACTH drive required to maintain plasma cortisol concentrations.

It seems most likely that the weak fetal zone androgens are responsible for the virilisation. This cannot be the only cause however because both we (see table) and others[4 5] have observed female preterm infants with raised concentrations of 3β-OH-5-ene steroids, who were not virilised. There may be other factors involved, for example variations in end organ sensitivity, localised conversion of fetal steroids to more potent androgens at the site of action in the genitalia, or high concentrations of circulating free androgens. Another factor may be the degree of immaturity of the preterm infant. Grumbach and Ducharme have pointed out that the virilising effect of an androgen depends on the maturity of the fetus. Androgen exposure between 8–13 weeks' gestation causes labial fusion and clitoromegaly, whereas after this time only hypertrophy of the clitoris is seen.[1] The fact that the most severely affected infant was 24 weeks' gestation may be a manifestation of the different response of the immature fetus to weak androgens. The increasing survival of such immature infants is a recent phenomenon and this may be the reason why clitoromegaly has not been reported before in preterm babies.

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Topical prostaglandin E_2 gel for cervical ripening and closure of the ductus arteriosus in the newborn

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Abstract

The closure time of the ductus arteriosus was investigated in 29 full term babies born vaginally after induction with prostaglandin E_2 and in 22 controls. Serial Doppler echocardiography studies showed a significantly prolonged closure time in babies induced by prostaglandin E_2. Whether the difference is related to changes in fetal prostaglandin E_2 concentration remains to be established.

In sheep prostaglandin E_2 given intravenously to the ewe can gain access to the fetus and cause fetal hypertension and vasoconstriction of the fetal renal, placental membrane, and umbilical circulations.[1] In pregnant woman local instillation of prostaglandin E_2 is being increasingly used to ripen the cervix before induction of labour. It is desirable therefore to find out whether vaginal administration of prostaglandin E_2 affects haemodynamic function in the fetus during the perinatal period. This study reports maternal and fetal blood concentrations of prostaglandin E_2 after local application, and compares the closure times of the ductus arteriosus in babies born after induction of labour with prostaglandin E_2 with those in a control group born by spontaneous delivery.

Patients and methods

Fifty one babies born at full term by vaginal delivery were studied. Twenty nine babies (mean (SD) gestational age 39·86 (1·66) weeks) were born to mothers who had received a 3 mg prostaglandin E_2 pessary (Upjohn) to ripen the cervix before induction of labour. Twenty two babies (mean (SD) gestational age 39·82 (1·14) weeks) whose mothers did not receive prostaglandin E_2 acted as controls.

DETERMINATION OF DUCTAL CLOSURE TIME

Serial Doppler echocardiography studies were done with an Aloka SSD-280S ultrasound scanner within 12 hours of birth, then twice daily at intervals of about 12 hours until ductal shunting could no longer be detected.[2]

MEASUREMENT OF MATERNAL AND CORD PROSTAGLANDIN E_2 CONCENTRATIONS

Cord blood was drawn from the umbilical vein immediately after clamping and cutting of the cord, and before placental separation. Paired maternal venous blood samples were collected at the same time. The samples were then transferred into prechilled polypropylene tubes containing EDTA and indomethacin, and cen-

Concentrations of prostaglandin E_2 in maternal and cord blood in patients treated with prostaglandin E_2 and controls

	Group treated with prostaglandin E_2		Control group	
	No of samples	Mean (SD) concentration (pg/ml)	No of samples	Mean (SD) concentration (pg/ml)
Maternal blood	23	139 (88)	17	109 (54)
Cord blood	20	298 (243)	16	292 (169)

There was no significant difference between the groups.

trifuged immediately at 2000 *g* for 15 minutes at 4°C. Plasma was separated and stored in aliquots at −70°C until assay.

A commercial kit for the measurement of prostaglandin E_2 by radioimmunoassay (Amersham) was used.[3]

STATISTICAL METHODS

Because the exact time of closure of each ductus arteriosus was not known, a generalised Kaplan–Meier method was applied to compare the times between the study and control groups.[4] The prostaglandin E_2 concentrations of the mother and cord blood in the study and control groups were compared by Student's *t* test. Multiple logistic regression analysis was used to calculate whether cord blood concentrations of prostaglandin E_2 and birth weight were factors affecting ductal closure time.

Results

Mean birth weights of the treatment and control groups were similar, being 3246 g (range 2500–4200) and 3317 g (range 2150–4500), respectively. The mean time interval between application of the gel and delivery was 13 hours (range 3 to 28).

CLOSURE TIME OF THE DUCTUS ARTERIOSUS

In the treated group half of the ductus arteriosuses closed at 28 hours compared with 15 hours in the controls. The difference was significant ($p<0{\cdot}05$).

PROSTAGLANDIN E_2 CONCENTRATIONS IN BLOOD

The results are shown in the table. There were no significant differences in the prostaglandin E_2 concentrations between the control and treatment groups either in maternal or cord blood. No correlation between the maternal and cord blood concentrations was seen in the control group, but a significant correlation was noted in the treatment group ($r^2=0{\cdot}39$, $p=0{\cdot}03$).

Multiple logistic regression analysis showed that birth weight and cord blood prostaglandin E_2 concentration are not significant variables when predicting whether the closure time of the ductus arteriosus would be shorter or longer than 24 hours (p values 0·06 and 0·21, respectively).

Discussion

The results of this study show that there was delay in closure time of the ductus arteriosus in newborn babies whose mothers had had prostaglandin E_2 pessaries for ripening of the cervix before the onset of labour. No clinical problems resulted, however. Whether delayed ductal closure also occurs in premature babies whose mothers are given prostaglandin E_2 deserves further investigation, especially as patent ductus arteriosus causes so many problems in preterm babies.

Maternal and cord blood concentrations of prostaglandin E_2 were, unexpectedly, not significantly higher in the treated group, but this might be because of the time that the blood was sampled. It has been reported that after vaginal administration of 3 mg prostaglandin E_2 in patients during weeks 9–14 of pregnancy, peak plasma concentrations of between 3500 and 10 000 pg/ml were observed three to six hours after the start of treatment; these returned to normal after 10 hours.[5] Whether the same dose of prostaglandin E_2 given to pregnant women at full term results in similar plasma concentrations is unknown. The low maternal and cord blood concentrations in our study, however, may be related to the interval between application of the gel and delivery (about 13 hours). Repeated maternal and fetal blood sampling at a predetermined time after the prostaglandin E_2 has been given may shed light on the relation between maternal and fetal blood concentrations.

We thank Professor DP Davies for his encouragement and help in the preparation of this paper.

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Paradoxical bone mineralisation in the twin to twin transfusion syndrome

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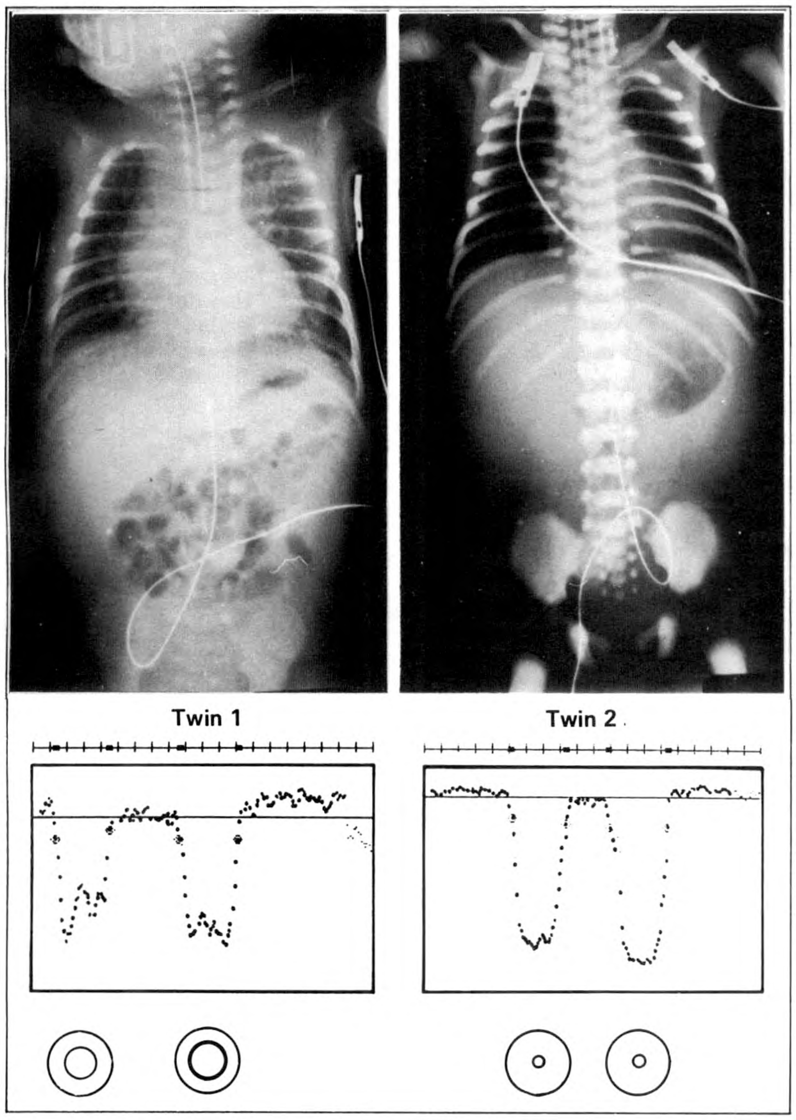
Abstract

We report twin preterm infants with the twin to twin transfusion syndrome, exhibiting grossly different bone densities on chest radiographs. Photonabsorptiometry showed the polycythaemic twin was osteopenic and the anaemic twin osteosclerotic; bone mineral contents were 0·028 g/cm and 0·074 g/cm respectively (normal mean (SD) 0·041 (0·006) g/cm. We speculate that alterations in macrophage derived osteoclastic activity contribute to these previously unreported findings.

The twin to twin transfusion syndrome is a well recognised clinical entity, occurring in approximately 7% of twin pairs.[1] An asymmetry of blood flow from the common placental bed, possibly in association with vascular stenosis,[2] results in polycythaemia in one twin and anaemia in the other, with a significant difference in weight at birth of more than 20% of the weight of the larger infant.



Twins 1 and 2: initial radiographs, scan pictures, and extrapolated cross sectional bone/profiles.

The mortality of such twin pairs is high and long term morbidity increased, with reduced non-verbal ability at follow up for the smaller twin, where intrapair weight birthweight differences are large.[3]

There is very little information available regarding the disturbances in metabolism likely to accompany the profound haematological inequalities between the infants, and we report here the evidence for a significant alteration in bone mineral homoeostasis in this condition.

Case reports

Twin girls were delivered at 31 weeks' gestation by emergency caesarean section, the indications being maternal pre-eclampsia and fetal distress. Twin 1 weighed 1972 g, and was plethoric (haemoglobin concentration 249 g/l, white cell count $24·3\times10^9$/l, macrophages $2·2\times10^9$/l, and packed cell volume 0·88). Twin 2 weighed 1591 g and was pale (haemoglobin concentration 49 g/l, white cell count $5·0\times10^9$/l, macrophages $0·93\times10^9$/l, and packed cell volume 0·18). Both infants required intubation at birth, and were ventilated to age 12 hours. The polycythaemic twin received two dilutional exchange transfusions with plasma, and the anaemic twin a single partial exchange with packed red blood cells.

Differences in bone density were noted on routine chest radiographs, twin 1 having osteopenic and twin 2 sclerotic bones. The growth plates appeared normal in both twins, and there were no subperiosteal resorption pits or fractures. Further investigations of bone mineral content were carried out using a Lunar SP2 (Lunar Radiation Corporation) photonabsorptiometer, on the left forearm at the 1/3rd distal site. Anthropometry comprised crown-heel length to the next succeeding 1 mm using a Holtain Neonatometer, weight to the nearest 10 g using Secca electronic balance scales, ulnar length to the next succeeding 0·1 mm using RS Precision Vernier Calipers, and occipitofrontal circumference to the next succeeding 1 mm using a paper tape measure.

The infants were scanned and measured initially at age 1 week, and followed up at 6 weeks actual postnatal age, and at 3 and 9

Bone mineral content and anthropometry

Age		*Length (cm)*	*Ulnar length (mm)*	*Weight (g)*	*Occipitofrontal circumference (cm)*	*Bone mineral content (g/cm)*	*Bone width (cm)*
1 Week	Twin 1	45·0	66·3	1656	28·7	0·028	0·422
	Twin 2	42·0	62·9	1425	28·1	0·074	0·424
10 Weeks	Twin 1	51·6	79·5	3380	34·8	0·041	0·496
	Twin 2	47·8	71·6	2960	35·4	0·054	0·440
3 Months	Twin 1	57·0	82·3	4700	39·5	0·080	0·632
Corrected	Twin 2	57·0	81·4	4800	40·9	0·072	0·569
9 Months	Twin 1	70·4	104·2	8160	46·0	0·123	0·655
Corrected	Twin 2	70·9	100·8	8250	46·8	0·131	0·702

months corrected age. The initial radiographs, scan pictures, and extrapolated cross sectional bone profiles are shown in the figure; the anthropometry and scan results are given in the table.

There were significant initial differences in crown-heel length and single bone (ulnar) length, in additional to the difference in birth weight. The bone mineral content estimation of 0·028 g/cm for the larger twin was low (normal at this postconceptional age is mean (SD) 0·041 (0·006) g/cm),[4] and that of the smaller twin substantially increased at 0·074 g/cm, a 2·5 fold difference overall.

The inequalities in mineral homoeostasis were further illustrated by biochemical measurements. Twin 1 had a progressive rise in plasma calcium concentration from birth, reaching 3·2 mmol/l at age 1 week, with a low plasma phosphate of 0·75 mmol/l, and raised plasma alkaline phosphatase activity (668 U/l, adult normal range <120 U/l) at that time. Twin 2 demonstrated a normal decrease in plasma calcium to 1·83 mmol/l at 24 hours age, followed by an increase to 2·24 mmol/l at one week. Plasma phosphate concentration at 1 week was raised (2·0 mmol/l) and plasma alkaline phosphatase activity within the range normally observed in preterm infants (315 U/l).

Discussion

These twins are the first reported cases of abnormal bone mineralisation in the twin to twin transfusion syndrome. The radiological, absorptiometric, and biochemical abnormalities observed represent a profound disturbance of mineral homoeostasis in both infants. The biochemical data for twin 1 are suggestive of hyperparathyroidism; however, apart from the osteopenia, the radiological evidence for this is slender, and it is also unlikely that the opposite defect would be present in the other twin.

At follow up, the bone mineral content and anthropometric measurements for each twin tended to a mean appropriate for their post-conceptional and postnatal ages, indicating a resolution of their common problem.

The cells responsible for the resorption of bone mineral are osteoclasts,[5] which are multinucleated giant cells produced by the fusion of activated macrophages. Reduced osteoclastic activity has been identified as the cause of osteopetrosis,[6] with the different forms of the disorder reflecting specific enzymic or maturational defects. The gross appearance of the bones in this disorder is similar to that observed in twin 2. Overactivity of osteoclasts, as occurs in hyperparathyroidism, leads to greatly increased bone mineral resorption, with reduced cortical thickness, as observed in twin 1.[5]

We speculate that reduced availability of macrophages for activation and transformation to osteoclasts in the anaemic twin, with the reverse situation for the plethoric twin, resulted in the changes observed here, the problem resolving as normal populations of osteoclasts were established in each infant postnatally.

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Linear growth in the early neonatal period

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Abstract

To test the hypothesis that early linear growth is independent of changes in weight we undertook simple anthropometry in 45 term infants daily to day 7 after birth. Linear growth proceeded rapidly and independently of changes in weight variations from the first day after birth; we suggest that this implies 'programmed' continuity of skeletal growth, possibly fuelled at the expense of other body tissues.

The pattern of rapid weight loss, followed by steady weight gain in the first days after birth is well reported.[1] Information documenting changes in linear growth at this time is sparse, however, possibly due in part to the technical difficulties inherent in such measurements.[2]

We wished to test the hypothesis that changes in early linear growth occur independently of alterations in weight in the early neonatal period, as part of a larger series of studies of body composition and energy metabolism during this period of rapid adaptive change.

Subjects and methods

We studied a total of 45 infants of appropriate size for gestation (mean (SD) birth weight 3370 (420) g) born to mothers attending the Rosie Maternity Hospital, Cambridge, for their delivery.

Where possible (38 of 45) we selected infants of mothers who had undergone caesarean section, in order to ensure at least five days' measurements. The reasons for operative delivery were previous caesarean section, cephalopelvic disproportion, or failure to progress in labour.

The anthropometry comprised crown-heel length to the next succeeding 1 mm using a Holtain neonatometer; ulnar length (distal ulnar styloid to olecranon) to the next succeeding 0·1 mm using a pair of RS precision vernier callipers; weight using Sartorius electronic balance scales to the nearest 10 g; and occipitofrontal circumference to the next succeeding 1 mm using a paper tape measure. All measuring devices were checked against standards daily, and recalibrated where necessary. Weight was obtained at birth; each of the other measurements was made within the first 24 hours, and on successive days thereafter.

The precision (standard error of measurement) for crown-heel length was 0·7 mm (n=33), and for ulnar length 0·2 mm (n=33). Interobserver variability was 2 mm for crown-heel length (n=21); only one observer carried out ulnar length measurements.

Thirty eight infants were breast fed, with four of these receiving 'complementary' formula feeds for one day each.

Results

The results are displayed graphically in figs 1 and 2. The graphs represent the mean incremental changes over successive 24 hour periods for each of the measurements performed. The points are for the group mean over that time period; the error bars are 1 standard error of that mean. No significant differences were

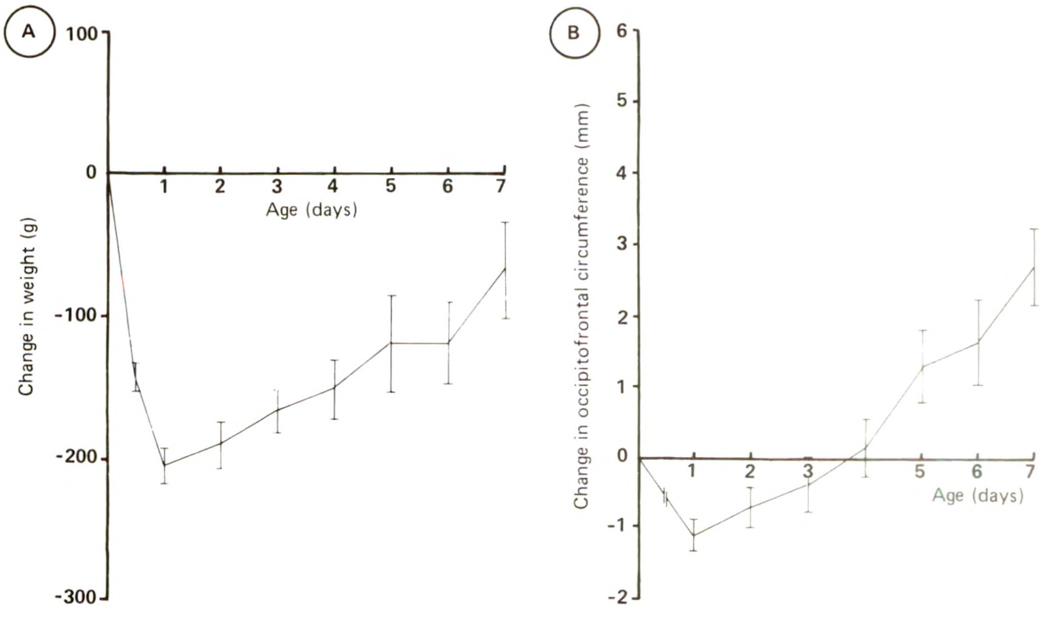

Figure 1 Change in weight (A) and occipitofrontal circumference (B) compared with age.

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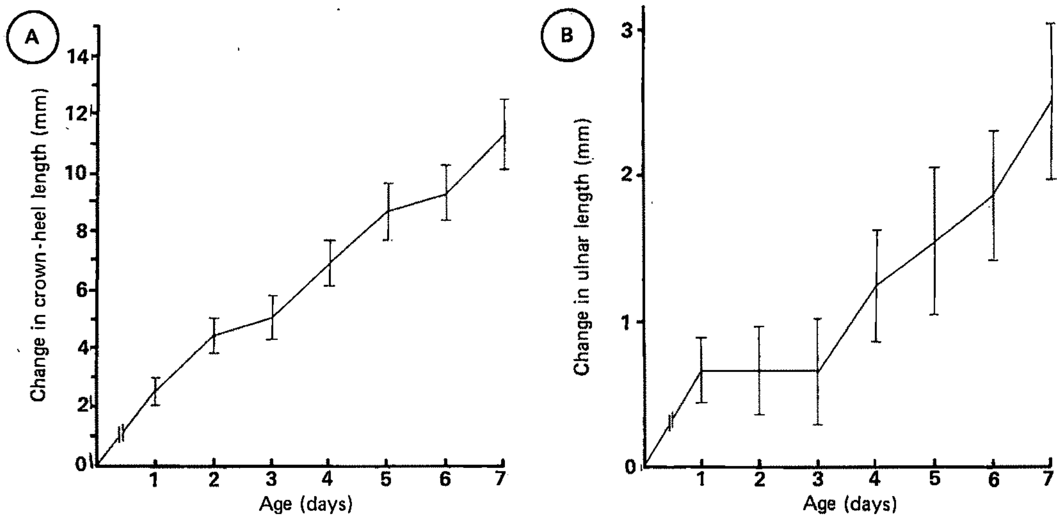

Figure 2 Increase in crown-heel length (A) and ulnar length (B) compared with age.

observed between the sexes, or between feed groups, and the data have therefore been combined.

As crown-heel length, ulnar length, and occipitofrontal circumference were not measured at the time of birth, the graphs for these measurements do not intersect the y axis. Their incremental changes are shown relative to the first measurement, made at a group mean age of 16 hours after delivery.

It can be clearly seen that despite losing weight, and with an initial decrease in head circumference (possibily due to moulding), both crown-heel and ulnar length increased from the first day after birth.

Discussion

We have shown that the linear growth of a single bone and of length overall continues from the first day after birth. The percentage increase in crown-heel length was 2·5%, and in ulnar length 3·0%. The close degree of agreement between these values makes it unlikely that other factors, such as variation in muscular tone, or alterations in soft tissue water content contributed significantly to the observed increase in body length.

The actual rate of growth of 11·5 mm in one week is the most rapid seen ex utero, and is essentially a direct continuation of that observed in the latter part of the third trimester.[3] By contrast, weight falls immediately after birth by an average of 6%, and is not usually regained by the age of 1 week. This implies that skeletal growth is to some extent 'preprogrammed' to continue despite the abrupt alteration in nutrient supply. It is possible that the consequent demand for mineral substrate contributes to the hypocalcaemia commonly observed in the neonatal period.[4]

Total energy requirements increase postnatally,[5] yet until enteral food intake is established there is negative energy balance. It is interesting that rapid early bone growth is given priority at this stage and is presumably fuelled at the expense of other tissues. Our observations also have important implications for the interpretation and collation of growth and mineral balance data in neonates.

The authors would like to thank Sister S Churchill for her assistance with anthropometry.

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Neonatal cilia: ultrastructure

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Abstract

The number of inner and outer dynein arms and gross cilial abnormalities of well newborn children and adults were similar. Inability to see all nine outer dynein arms in healthy adults may be due to technique and not recent infection or congenital cilial malformation.

Inner and outer dynein arm counts and percentage of multiple or disorganised cilia and cilia with missing doublets in adults and neonatal patients

Subject No	*No of cilia counted*	*Outer dynein arms*	*Inner dynein arms*	*Multiple (%)*	*Disorganised (%)*	*Missing doublet (%)*
Adults						
1	35	6·3	2·3	—	—	—
2	30	7·3	3·7	—	—	0·1
3	26	7·6	3·5	—	2·7	2·2
4	35	8·1	3·6	2·1	—	6·3
5	46	7·2	2·9	2·5	2·5	10·6
6	61	6·6	3·4	—	—	—
7	42	6·5	3·0	—	—	—
8	36	6·5	3·2	0·7	1·5	—
9	29	7·2	4·3	—	—	0·6
10	46	7·5	3·3	—	—	10·6
Neonates						
11	38	6·8	3·3	1·6	1·7	0·0
12	15	6·7	3·8	2·6	3·7	1·1
13	38	7·9	3·4	—	0·3	2·7
14	26	6·9	4·2	2·8	1·0	4·0
15	29	6·8	3·7	—	0·3	—
16	36	7·1	3·2	—	—	—
17	30	5·8	3·3	—	—	1·3
18	18	6·9	3·3	—	2·3	0·8
19	33	6·0	3·6	—	—	—
20	41	5·1	3·2	—	0·6	—
21	48	7·8	4·2	1·8	—	—
22	32	7·1	3·7	—	—	1·9
23	28	6·2	4·2	—	2·6	—
24	38	7·2	2·8	1·0	1·8	1·2
25	37	7·8	3·2	1·8	—	0·6
26	40	6·0	2·4	—	4·2	8·0
27	25	6·7	2·1	1·0	4·1	12·3
28	36	6·8	3·7	—	—	—
29	35	6·8	3·8	2·7	5·3	10·0
30	32	5·5	3·4	—	0·0	0·0

Respiratory tract infections are common in patients with abnormal nasal cilial structure.[1] The presence of genetically abnormal cilia has prognostic implications and the study of ciliary structure is a useful investigation in children with recurrent respiratory tract infections.

Cilial ultrastructure is assessed by determining the mean number of inner and outer dynein arms in a large sample of cilia. In addition, abnormalities such as multiple, disorganised cilia or cilia lacking microtubules are noted.

It is rarely possible to see all inner and outer dynein arms in a cilial sample probably because of recent infection or fixation technique. We compared cilial ultrastructure in a group of adults with well newborn children before any respiratory illness to determine the effect of fixation on electronmicroscopical appearance.

Patients and methods

Twenty well neonatal patients from the postnatal ward were studied; their mean (SD) age was 1·8 (0·5) days. Ten adults with mean age of 21 (5) years, and who had been free from an upper respiratory tract infection for at least six weeks, were studied.

Cilia were obtained by nasal brushing. In adults this was done by direct vision of the inferior nasal turbinate bone. In the neonatal patients the brush was inserted 1·5 cm, rotated, and withdrawn in a blind fashion from the nose.

Immediately after brushing the cilia were dislodged by brisk agitation directly into sodium cacodylate buffer at pH 7·4 and fixed in buffered glutaraldehyde. Ultra thin sections were prepared and stained with urynl acetate and lead citrate. The grids were examined by transmission electron microscopy (×75 000) and gross structural abnormalities recorded. These included the percentage of cilia with missing doublets and percentage of multiple or disorganised cilia. Magnification was raised (×130 000) and the number of dynein arms counted (>150 individual cilia from each patient). A tilting and rotating grid holder allowed optimal cross sectional cilial views.

An unpaired *t* test was used for statistical analysis. Ethical approval was given by the Nottingham University Hospital Committee and parental consent was obtained.

Results

There was no significant difference in the number of gross abnormalities (percentage of multiple or disorganised cilia or percentage of cilia missing doublets) or numbers of inner and outer dynein arms between the neonatal or adult groups (table). No neonatal patient appeared to be distressed by the nasal brushing. Several sneezed but none had a nose bleed.

Discussion

It is rare to see all of the inner and outer dynein arms of a cilia. It has been difficult therefore to say whether the reduced number of dynein arms seen is due to fixation, infection, or congenital absence. Older children and adults are frequently exposed to upper respiratory tract pathogens that may induce changes in nasal cilia.[2, 3] Some of these changes last for short periods of time, but others may take several months to disappear after treatment of the precipitating cause.

The neonatal patients we studied were well and most less than 3 days old, thus we assumed their cilia were normal. There was no difference in cilial ultrastructive between adult controls and the neonatal group. Thus with our technique for measuring dynein arms we can assume the mean (SD) value of 6·81 (0·7) for outer arms and 3·38 (0·54) for inner arms in the adult group is due to fixation and technique and not loss of dynein arms with age or insult. It is important that electronmicroscopists define the normal values as this may vary from unit to unit. In addition, full cilial analysis includes functional studies as ultrastructure may be normal but function abnormal.[1]

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PERINATAL LESSONS FROM THE PAST

Charles-Michel Billard (1800–1832): pioneer of neonatal medicine

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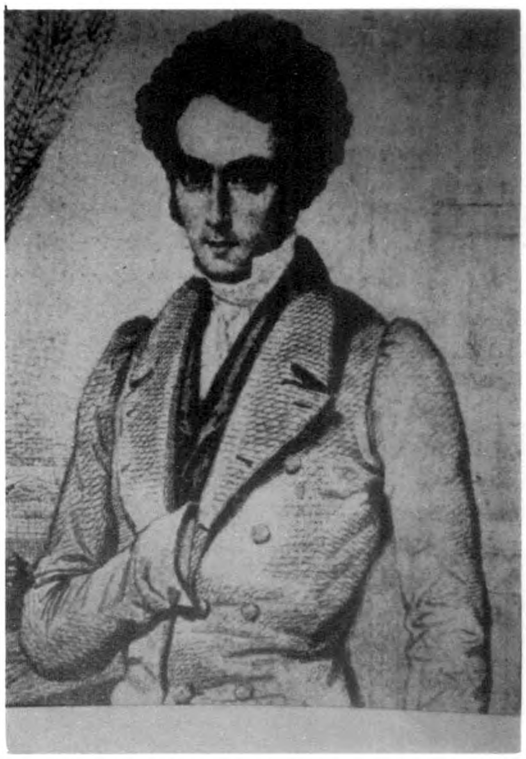
Billard was born in Pellouaille, France, in 1800. He studied medicine, first in Angers and then, between 1824–8, in Paris. During this time he was very poor and supported himself by writing for medical journals and undertaking translations, having learned German, English, and Italian. In 1828, the year he became Docteur en Médecine de la Faculté de Paris, he published his masterpiece *Traité des Maladies des Enfants Nouveau-Nés et à la Mamelle*. Sadly this brilliant and modest man then contracted phthisis and returned with his wife and child to Angers where he died at the early age of 32.

Billard's book was the first systematic clinical/pathological text on the newborn infant. Indirectly the fetus was also studied; for, as he wrote:

> 'During intrauterine life man often suffers many affectations, the fatal consequences of which are brought with him into the world . . . children may be born healthy, sick, convalescent, or entirely recovered from former diseases'.

His experience of newborn infants was based on a year working at the Hospice des Enfants Trouvés in Paris. During that year, 1826, 5392 infants were admitted to the hospital, of which 1404 (26%) died. In passing, it is of interest that Billard mentions that the average weight of the term infant at that time was between 5–5½ lb. He describes his clinical/pathological method of approach in the introduction to his book:

> 'One day in reading Morgagni, a favourite author of mine, I was particularly struck with a passage in the latter part of his forty-eighth epistle. This distinguished observer, after having enumerated the affections to which newborn children are liable, complains of the little progress that has been made in their pathology, and laments with much feeling that mothers, from a false tenderness, constantly oppose the examination of the bodies of their children, the symptoms of whose diseases may have been watched with great carefulness and attention. "How vast and new", says he, "is the space that is still open before us for the study of the diseases of young children!"
>
> Occupying for a year an important station in the Hospice des Enfants Trouvés, in Paris, I observed with close attention the children in that institution and upon the occurrence of a fatal termination of their diseases, I availed myself of the opportunity thus afforded to examine by dissection all their organs, and ascertain the causes and seat of each disease. In this manner has the wish of Morgagni been fulfilled. I have been able by these means to compare the symptoms observed during life with the anatomical lesions by which they have been produced'.



Charles-Michel Billard of France, 1800–1832.

For the English text of Billard's work we are indebted to Dr James Stewart's translation of the third edition (1837).[1] The 545 pages are packed with original descriptions and insights which in many instances have been credited to others since his time. The approach is so modern that it may be read with delight and value by anyone with an interest in perinatal medicine. The following few extracts may stimulate others to explore and enjoy this great work:

On a case of congenital urethral atresia

'In this child it appears that the obliteration of the urethra caused the dropsy of the bladder, and the latter, the hydropic affection of the kidneys, the normal development of which was hindered or even suspended . . . This case will serve to prove that the excretions of the foetus, at least those of the urinary organs, are, in the normal condition, rejected from the body, and probably are mixed with the waters of the amnios, since it appears that when any obstacle occurs to the passage of this fluid, it reflows into the reservoirs, and distends them excessively, in the same manner as is observed in adults who are affected with stricture of the urethra, or paralysis of the bladder. This remark ought to have a place in the history of embryology'.

On nasal respiration

'It may create some surprise to see the nasal fossae classed with the organs of the respiratory apparatus. In man, the nose and the nasal fossae are but an accessary part, and one not immediately dependent on the respiratory system; they are especially destined to olfaction. In some animals, and particularly among fishes, the nasal fossae are altogether separated from the respiratory passages; but this is not the case with an infant at birth; it respires very little by the mouth, which is almost always shut, and while sucking, it is essentially necessary that the air penetrate the lungs through the nasal fossae, since the buccal cavity, applied to the nipple, is continually filled with milk. Besides, without this it would be impossible to prolong the act of sucking for any length of time'.

On primary atelectasis

'It is not usual to meet with so complete an absence of air in the lungs of infants, who, like the one just mentioned, live for some time, as it were, the embryo life, either because the blood preserves its vivifying principle which it received from the mother, or that the oxygen of the air absorbed by the skin or mucous membranes penetrated into the circulation, or that this essential element of life is not at this time of so great importance as it is afterwards'.

On imperfect establishment of respiration

'. . . the air sometimes passes freely into the lungs at the period of birth, but the sanguineous congestion which occurs immediately expels it or hinders it from penetrating in sufficient quantity to effect a complete establishment of life. There exists, as is well known, between the circulation and respiration, an intimate and reciprocal relation, which is evident during life, but more particularly so at the time of birth . . . The symptoms of pulmonary engorgement in an infant are, in general, very obscure, and consequently difficult of observation; yet we may point out the following: the respiration is laboured; the thoracic parietes are not perfectly developed; the face is purple; the general color indicates a sanguineous plethora in all the organs; the cries are obscure, painful, and short; percussion yields a dull sound'.

On jaundice of the brain

'I have remarked the yellow colouring which constitutes jaundice, in four instances, in the brain and spinal marrow; the brain, which was of moderate firmness, presented a uniform and bright yellow in two of these subjects, while the color was in isolated patches in the other two. In three of these cases the substance of the medulla was of a deep yellow, and its consistence very soft; and in the two subjects where the yellow color of the brain was uniform, there existed, at the same time, a general jaundiced affection of the skin'.

Of the attitudes of the child

'If the child, immediately after birth, and while the accoucheur is engaged in tying the umbilical cord, be allowed to remain in the situation in which it was expelled from the uterus, it will be observed that a flexion of its members takes place, the head and breast approximate, the body rolls upon itself, and assumes a posture somewhat similar to that it had while within the womb . . . In a word, the flexed position of the limbs and the forward curvature of the trunk, constitute the peculiar position of a newly born infant'.

Of the colour of the skin

'Infants recently born, are almost all of the same color. Blood predominates in their tissues and communicates, to them its hue and the face, body, and limbs are all strongly colored . . . If it continues, it is not so intense as at first; it becomes of a violet hue, and the hands and feet more particularly exhibit this change of color . . . When the finger is applied to the skin of an infant, the red color disappears at this point, and it becomes yellowish; afterwards the blood returns by degrees in the capillaries from which the pressure had removed it, and the yellow tint is replaced by the previous red. It will often be observed, after the red color has disappeared, and before it becomes altogether white, that the skin will exhibit a universal tint of yellow, and sometimes of a copper color. This is thought, by physicians generally, to indicate an affection of the liver'.

---

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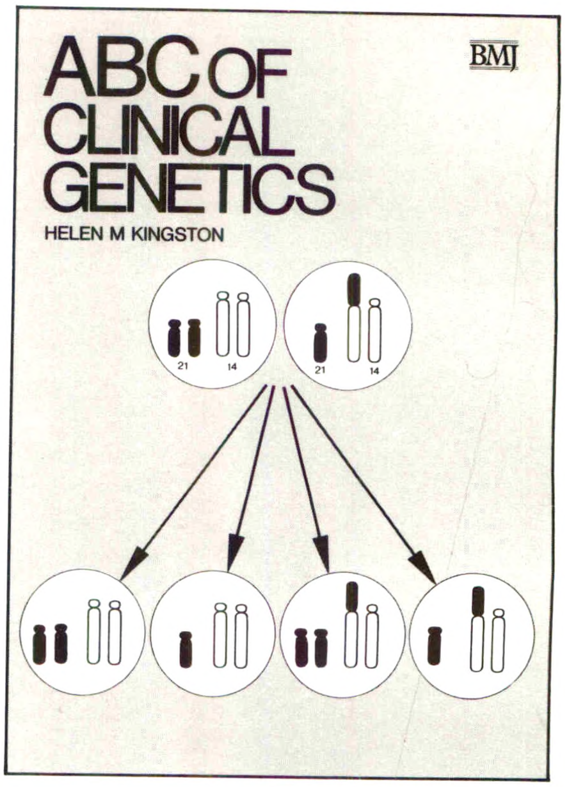

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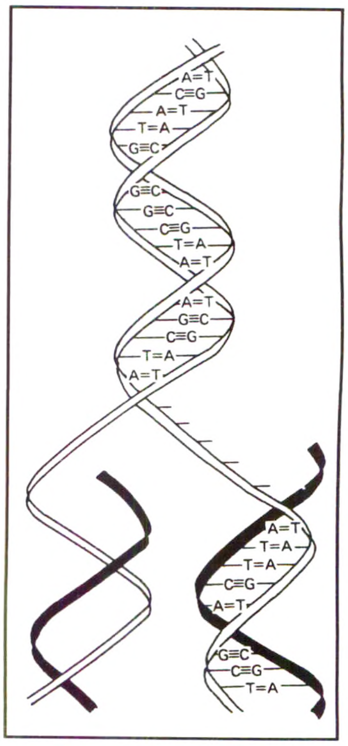

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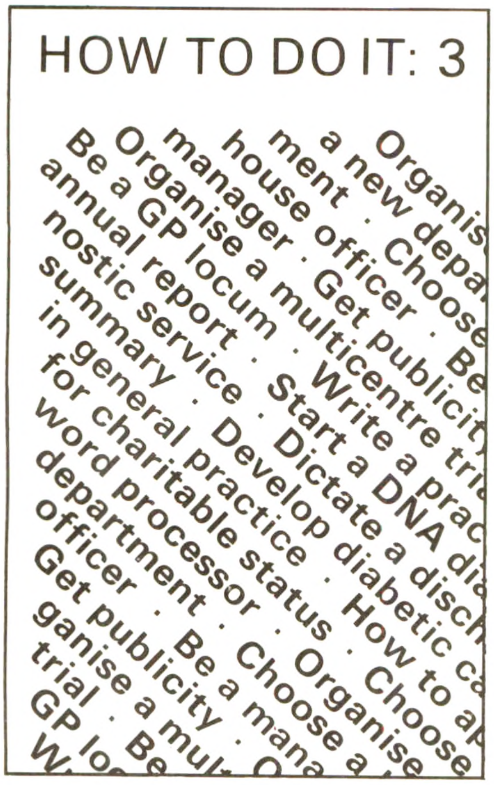

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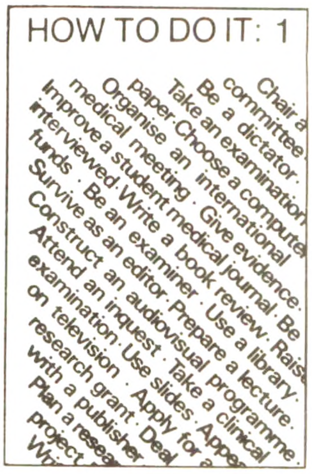

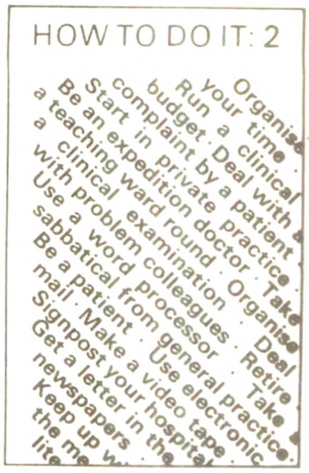

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Annotations

Genomic imprinting

Over the last few years a compelling body of evidence has accumulated indicating that genomic imprinting must be occurring in parts of the human genome.[1] The concept of genomic imprinting is difficult for people who have grown up with the concepts of mendelian inheritance. In general, the question has not been asked whether a particular gene has a different outcome when it is inherited from the parent of one sex as compared with when the same gene is inherited from a parent of the opposite sex. The concept of genomic imprinting implies there are differential modifications of genetic material, either chromosomal or single gene, depending on whether the genetic material is passed on from the male or the female parent. This kind of modification of the nuclear DNA in the somatic cells is thought to lead to differential expression of the genetic material and therefore different phenotypes. However, this kind of modification is not permanent, not a mutation of the gene itself, not an allele of the particular gene, but rather a temporary change in the function of the genetic material, albeit perhaps for the lifetime of the individual.

The evidence which has been accumulated from many different areas of research suggests very strongly that imprinting effects must occur in some parts of the human genome. At the present time, there are seven different kinds of observations that suggests the presence of genomic imprinting. Some come from studies in mice and some from studies in humans. These studies include the observations of nuclear transplantation—for example, parthenogenetic work in mice by Solter[2] and Surani,[3] in which zygotes are constructed so that all the nuclear genes (that is, both sets of haploid chromosomes) are entirely derived either from the male or female parent. These reconstituted zygotes each have a complete set of chromosomes, but a set of chromosomes that has been derived entirely from only one type of parent. When these zygotes are allowed to develop they have strikingly different phenotypes suggesting that maternally and paternally derived genetic information has different roles.

A second piece of evidence comes from human triploid work by McFadden and Kalousek (D McFadden, D Kalousek, personal communication) in which a triploid with two paternal and one maternal complement is compared with a triploid with two maternal and one paternal complement. They have quite different phenotypes with the paternal complement contributing more to the placenta and the maternal complement to the embryo.

The third type of evidence comes from uniparental disomy work in mice by Searle and Beechey,[4] Lyon and Glenister,[5] Cattanach and Kirk,[6] and others in which mice are constructed using translocation chromosomes such that both copies of a specific chromosome segment have come from one parent. Thus these mice have a balanced set of chromosomes but both copies of a particular segment will have come from only one parent. In the case of eight (and maybe nine) mouse chromosome segments there are appreciable phenotypic differences on growth behaviour and survival when both copies of a specific mouse chromosome segment are derived from one parent. There are at least two situations in humans in which there is uniparental disomy (that is, both copies of a chromosome have been shown to come from only one parent) for a specific chromosome and both have been associated with changes in growth and behaviour. One of these situations is found in cases of maternal uniparental disomy with the patient having cystic fibrosis.[7 8] Both reported cases had intrauterine and postnatal growth retardation. There are many alternative explanations, but the homologous chromosomal disomy in mouse also has intrauterine growth retardation. The second situation in humans is found in the reported case of Prader-Willi syndrome in which there were two chromosomes 15 from the mother and no deletion, but no paternal 15.[9] It appears that the absence of the paternally derived chromosome 15 produces typical Prader-Willi phenotype.

The fourth type of disorder that indicates that there are differences between maternally compared with paternally derived segments of chromosomes are the chromosome deletion syndromes such as Prader-Willi and Angelman's syndromes where there appear to be dramatic differences in the parental derivation of the deleted chromosome.[10 11] There are similar if not identical chromosome deletions of chromosome 15 in both conditions; however, in Prader-Willi syndrome there has always been a deletion or absence of the paternal chromosome 15. Most cases of sporadic Angelman's syndrome are associated with a deletion of chromosome 15 and it is always the maternally derived 15. These findings imply that part of the chromosome 15 that is inherited from the father is treated differently than the same part of chromosome 15 when inherited from the mother.

Work on congenital and infantile tumours suggests that a similar kind of phenomenon is occurring. These tumours are associated with loss of heterozygosity—that is, loss of predictable chromosome segment.[11] However, Wilms' tumour, retinoblastoma, and some sarcomas have dramatic differences in the parent of origin for the chromosome involved in the loss of heterozygosity. In cases of sporadic Wilms' tumour when there is loss of chromosome 11, it is almost always loss of the maternal chromosome 11.[12] Interestingly, however, familial Wilms' tumour is usually transmitted from the father and is specifically not linked to chromosome 11.[13] Thus there must be two or more genes with different roles involved in the production of Wilms' tumour, at least one of which has parental derivation effects.

With regard to the retinoblastoma gene, sporadic sarcomas (not those in the retinoblastoma patients) are frequently associated with chromosome 13 loss. Again, it is almost always the maternal chromosome 13 which is lost.[14] By contrast, in new unilateral and some sporadic bilateral retinoblastoma when there are mutations of chromosome 13 there does not seem to have been any particular parent of origin selection. In new germ line mutations for retinoblastoma, however, there is preferentially involvement of the paternal chromosome 13. These findings imply that tissue specific modifications of the retinoblastoma gene allow tumour development to occur first in a paternally derived gene, and then there is secondary loss of the tumour suppressing maternal gene (loss of heterozygosity).[15] Sporadic sarcomas act differently from sporadic retinoblastomas, however, suggesting that there may be tissue differences in the imprinting of the same gene.

The sixth kind of observation which suggests that there is differential modification of genes depending on the parent of origin are those made on transgenic mice in which something like 20% of transgenes expressed preferentially when inherited from one parent compared with the other.[16] Nonexpression is associated with methylation. In transgenic mice there is incorporation of the transgene DNA into the chromosome. The DNA does not change from generation to generation but the ability to express the gene does. Thus there will be different phenotypic effects depending on whether the gene is transmitted from the mother mouse or from the father mouse.

The last kind of observation which suggests that imprinting occurs is that made of specific genes. In humans the classic examples are Huntington's disease and myotonic dystrophy. In 5–10% of families with Huntington's disease, when the Huntington gene is transmitted from the father, there may be a severe rigid juvenile onset form. By contrast in 10–15% of families affected with myotonic dystrophy a severe congenital form may occur when the gene is transmitted from the mother. In addition, there are a number of other disorders in which more severe or earlier an onset or specific manifestations are observed when the gene is inherited from one parent compared with the other.[1]

Taken together these seven types of observations strongly suggest that genomic imprinting—that is, differential expression of maternally and paternally derived DNA—does occur in some parts of the human genome and therefore would be expected to play a part in human disease.

How can we identify these diseases? The first way is to be suspicious in the various classes of disorders, to ask the question whether there is earlier onset, more severe affliction, and multiple manifestations of disease when inherited from mother versus from father. In chromosomal abnormalities with deletions, duplications, and translocations we must ask the same kind of question.

From the mouse transgenic work and in human diseases observed thus far we would expect that an imprinting effect might *not* be absolutely constant. In other words, it may be something that only shows up in 10%, 15%, or 20% of families with a specific disorder. In mice, we call these strain differences in expression of imprinting, in humans we might expect there to be ethnic differences.

Another clue to finding imprinting in humans would be to anticipate what a pedigree in which imprinting was occurring would look like. Again, the term imprinting is used to describe a modification which does not allow expression. Thus in maternal imprinting there will be phenotypic expression or more noticeable expression when a gene is transmitted from the father but not when transmitted from the mother.[1] Paternal imprinting is the term used to imply that there is a phenotypic expression or more noticeable expression of the gene when transmitted from the mother. Because there will be phenotypic effects only when the gene or chromosome segment in question is transmitted from one or the other parents, there will be a number of non-manifesting transmitters. It is important to point out that there are equal numbers of males and females affected or non-affected phenotypically in each generation. A non-manifesting transmitter gives a clue to the sex of the parent who passes on the genetic information that could be expressed. In other words, in maternal imprinting there will be 'skipped' male non-manifesting individuals and in paternal imprinting there will be 'skipped' female non-manifesting individuals.

It is important as well to point out that in these pedigrees depending on what part of the pedigree is being examined, the pedigree can look like that of a dominantly inherited trait or a recessively inherited trait, or as a multifactorial disorder. Thus we need to re-examine pedigrees from multifactorial disorders and ask the question whether the expression has to do with the parent of origin. Similarly, there is a wealth of data to reanalyse from the clinics for common disorders such as sickle cell and Tay-Sachs diseases and phenylketonuria.

Another clue to potentially imprinted disorders are the homologous areas of human and mouse genome. Using the Oxford grid it is possible to define, with some accuracy, in humans where the homologous areas of DNA are mapped when compared with the mouse.[1] [10] Using the areas from the translocation disomy of mice that are involved in imprinting and transferring those areas to homologous areas of the human chromosomes, one can ask whether or not human disorders suspected of having imprinting phenomenon are located in the areas homologous to mouse 'imprinted' areas. Indeed we find that myotonic dystrophy, cystic fibrosis, Beckwith-Wiedemann syndrome, and many other human disorders are in mouse imprinted homologous areas. Angelman's and Prader-Willi syndromes are close to the mouse areas where very similar phenotypes are seen in the mouse disomies.[1]

Thus it behoves us all to re-examine specific parts of the human genome. One can look for a particular gene in a particular area or ask about genes related to a particular disease type process, for example one can examine the growth factors and endocrine genes in mouse homologous imprinted areas.[1] A number of very interesting diseases which have been described as having variable expressivity are present in those areas. Specific family trees need to be re-examined to see whether or not a pattern of expression is related to the sex of the parent of origin.

In summary, compelling evidence has been accumulated that some areas of the genome function differently depending on from which sex parent they are inherited. This process appears to be normally reversible and involves a temporary DNA modification. The differential function depends on which parent transmits the gene. This may be a ubiquitous phenomenon explaining a variety of observations that hitherto have been considered to be atypical, decreased penetrance, or to have inconsistent inheritance patterns. Heterogeneity, variable expression, and decreased penetrance are terms that have been developed to explain empiric observations. Reconsideration of family history, expression patterns, and disease processes may reveal that imprinting effects are much more common than we expected. It is clear that mammalian development requires the functional and complementary presence of at least parts of both maternal and paternal genome. Affects on embryonic and fetal growth and behaviour have been observed. The challenge now is to determine how many childhood and adult disorders also are associated with genomic imprinting.

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Neonatal infections with coagulase negative staphylococci

Staphylococci are members of the family of bacteria Micrococcaceae. They are Gram positive, catalase positive cocci that form grape like clusters (from the Greek staphyle: bunch of grapes, coccus: grain or berry). The classification of staphylococci is extremely complex and has been revised repeatedly over the last 30 years. More and more new species have been recognised, so that there are now 19 distinct species recognised by Bergey's *Manual of Systematic Bacteriology* (1986). The coagulase test is one of several tests used by clinical laboratories to distinguish *Staphylococcus aureus* from other staphylococci. 'Coagulase negative staphylococci' is a term used to describe species that do not coagulate plasma under the defined conditions of the coagulase test.

Coagulase negative staphylococci are frequent blood culture isolates from neonates in many intensive care units.[1 2] Neonatal infections with coagulase negative staphylococci are hospital acquired and are usually diagnosed after the first week of postnatal life.[3] Quantitative blood culture techniques have shown that the numbers of coagulase negative staphylococci in the blood of premature neonates with bacteraemia may exceed 1000 colony forming units/ml.[4 5] Defective opsonisation and phagocytosis may allow these large numbers of coagulase negative staphylococci to circulate.[6] *Staphylococcus epidermis* is the species most frequently associated with neonatal infection,[7] although other species such as *Staphylococcus hominis, Staphylococcus warneri,* and *Staphylococcus haemolyticus* have also been implicated. The strains involved in neonatal infection are usually resistant to a wide range of antibiotics.[8] Antibiotic resistant strains are spread from neonate to neonate on the hands of medical and nursing staff leading to colonisation of the skin of premature neonates in intensive care units within the first week of life.[9] Apart from the skin, another major reservoir of antibiotic resistant coagulase negative staphylococci is the bowel of neonates, where numbers may exceed 10^{10} colony forming units/g dry weight.

Many strains of coagulase negative staphylococci secrete a complex mucopolysaccharide,[10] which has been termed 'extracellular slime substance'. Electron microscopy studies suggest that it stabilises the attachment of coagulase negative staphylococci to the surfaces of foreign bodies such as intravascular catheters.[11] The influence of extracellular slime substance on the adherence of coagulase negative staphylococci to the immature skin of premature neonates is not known. Extracellular slime substance has been reported to have a number of immunomodulating effects such as inhibiting antibody binding to the staphylococcal cell wall, reducing the chemotactic response of neutrophils, and interfering with T and B cell function.[12] Although extracellular slime substance is probably important in stabilising the attachment of bacteria on surfaces, its importance in determining pathogenicity is controversial.[13] It is not produced by all clinically significant isolates of coagulase negative staphylococci and other bacterial surface characteristics such as bacterial cell surface hydrophobicity may be more important determinants of pathogenicity.[7 14]

It is not possible to sterilise the skin and therefore a proportion of percutaneously collected samples will be contaminated with cutaneous flora. Use of quantitative blood culture techniques may help to differentiate blood culture contamination from bacteraemia.[4 5] The proportion of blood cultures collected from neonates and children that are contaminated with coagulase negative staphylococci has been estimated at 7–10%.[5 15] This proportion is similar to that reported for blood cultures collected from neonates with necrotising enterocolitis.[16]

There is no evidence that coagulase negative staphylococci can cross intact skin. Intravascular catheters frequently become colonised with antibiotic resistant strains,[17] and neonatal bacteraemia with coagulase negative staphylococci is frequently associated with the use of intravascular catheters.[4 5 18] It is likely that infected catheters are the most common source of neonatal infection with coagulase negative staphylococci. The mechanisms by which bacteria colonise intravascular catheters are poorly understood. Bacteria may be washed into the catheter after contamination of infused fluids or migrate from the cutaneous surface along the outside of the catheter. The relative importance of these two routes of catheter colonisation in neonates is not known. In addition to catheter associated infections, coagulase negative staphylococci may cause wound infections and urinary tract infections after urological surgery. Meningitis with coagulase negative staphylococci in neonates is usually associated with ventricular drainage devices. The clinical signs of bacterial infection in neonates are non-specific. Rapid tests such as buffy coat examination[19 20] and tests for neutrophil activation or acute phase protein response[20–22] may improve the accuracy of diagnosis at the onset of episodes of suspected infection.

In contrast to Gram negative infections, neonatal mortality associated with infections caused by coagulase negative staphylococci is unusual. The clinical signs of infections with coagulase negative staphylococci are often minor with insidious onset of signs such as lethargy, increased frequency of apnoea, hypotonia, and poor peripheral perfusion. Delayed treatment may lead to disseminated disease, however,[18] and so treatment with an appropriate antibiotic should be initiated as soon as infection with coagulase negative staphylococci is suspected and the appropriate diagnostic samples have been taken. Isolates of coagulase negative staphylococci from neonates are often resistant to penicillin and aminoglycosides, but are also commonly resistant to a range of other antibiotics such as chloramphenicol, cloxacillin, and erythromycin. Unless specific tests are performed, resistance to β-lactam antibiotics may not be detected by the laboratory. Resistance of neonatal coagulase negative staphylococcal isolates to the glycopeptide antibiotics, vancomycin and teicoplanin, has not been reported and these are the antibiotics of choice for treating suspected neonatal infections with coagulase negative staphylococci. Strains of vancomycin resistant *S haemolyticus* were isolated from the peritoneal dialysis fluid of an adult.[23] Rifampicin inhibits a wide range of micro-organisms, including staphylococci at very low concentrations, but must be used with another antibiotic to avoid the rapid emergence of rifampicin resistance. Ventriculitis or meningitis associated with use of intraventricular drainage devices can be controlled with intravenous vancomycin combined with oral or intravenous rifampicin, but eradication of the infection usually requires removal of the colonised device. In neonates with suspected catheter associated infections, peripheral intravenous catheters associated with local signs of inflammation should be removed. Whether or not central lines colonised with coagulase negative staphylococci should be removed from neonates is controversial. There are no published comparative studies of treatment regimes for neonates with infected central lines.

The frequency of neonatal infection with coagulase negative staphylococci may be reduced by restricting the duration of use of intravascular catheters, avoidance of contamination of infusion system connections, better care of the catheter insertion site, and delegation of responsibility for the management of central lines to a specialist team. Better understanding of the mechanisms leading to bacterial colonisation of catheters and of the interaction between intravascular catheters and developing skin may lead to new strategies for preventing infections with coagulase negative staphylococci in premature neonates.

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ORIGINAL ARTICLES

Ischaemic brain lesions diagnosed at birth in preterm infants: clinical events and developmental outcome

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Accepted 29 March 1990

Abstract

In 232 newborn babies of <32 weeks' gestational age ultrasound brain scans were carried out shortly after birth, daily for the first week of life, and at least twice weekly thereafter. Periventricular echogenicity associated with or progressing to periventricular leucomalacia (ischaemic brain lesions) was present in nine babies within two hours of birth (early onset); 30 other babies subsequently developed similar lesions (late onset). Babies with early onset lesions were more likely to have a history of intrauterine growth retardation and to have recurrent apnoea in the first few hours after birth. By contrast, late onset lesions were associated with hyaline membrane disease. In later childhood four of six survivors with early onset periventricular leucomalacia, and seven of 14 survivors with late onset periventricular leucomalacia, had various disabilities including spastic diplegia, impaired vision, squints, sensorineural hearing loss, and developmental delay. In a third of the children with disabilities ischaemic brain lesions were noted within two hours of birth, raising the possibility that some of this damage had taken place before or during birth.

Certain clinical events that occur before or during birth may lead to ischaemic brain lesions and periventricular haemorrhage in newborn babies.[1 2] These include maternal antepartum haemorrhage, and perinatal hypoxia, hypercapnia, and acidosis in the baby. As the quality of ultrasound imaging has improved with the use of high resolution probes (7·5 MHz) areas of increased echogenicity consistent with brain ischaemia can be recognised with greater accuracy, and their progress to the development of cystic areas (periventricular leucomalacia) has been described.[3] Periventricular leucomalacia seems to occur more often in newborn babies weighing <1000 g and had been correlated with intrauterine infections, and placental vascular anastomosis in twins.[4] In a previous study we suggested that a reduction in systemic blood pressure associated with birth asphyxia, antepartum haemorrhage, recurrent apnoea, and septicaemia is responsible for the ischaemic brain lesions.[5]

In the present study we report various clinical events in babies in whom evidence of ischaemic brain lesions was present on the first ultrasound scan. We compared these findings with those in babies who subsequently developed similar brain lesions in our special care baby unit. Both groups of babies were followed up after leaving hospital and their developmental outcome assessed.

Patients and methods

Ultrasound brain scans were carried out in an unselected group of 232 newborn babies of <32 weeks' gestational age born at this hospital, from January 1984 onwards. The scans were carried out shortly after birth, daily for the first week of life, and at least twice weekly thereafter during their stay in the special care baby unit. A Technicare mechanical sector scanner with a 7·5 MHz probe was used and during the scanning special attention was given to the presence of areas of increased echogenicity consistent with ischaemic brain lesions (periventricular leucomalacia), periventricular haemorrhage, and ventricle size as previously described.[3 5] A record of each brain scan was kept on videotape. The accuracy of our findings on ultrasonography compared with those at necropsy was confirmed in a total of 53 babies who died during the neonatal period. The consistency was 85–90%, which is comparable with previous reports.[6] Clinical information was recorded prospectively for each baby.

FOLLOW UP

Babies with a history of periventricular leucomalacia were seen regularly in the outpatient clinic at a hospital near their place of residence, and information about their clinical progress was made available to us. They were also seen at this hospital at 18 to 48 months of age, when each child had a general medical and detailed neurological assessment according to our established protocol.[7] Development was assessed subjectively according to Illingworth,[8] at outpatient visits, and objectively by the Griffiths's scales of mental development in our department of child psychology for evidence of developmental delay (developmental quotient <80).[9] If a squint or visual impairment was suspected the child was referred to an ophthalmologist. Those children with hearing impairments were referred for detailed audiological testing.

STATISTICAL ANALYSIS

Mean values were compared using Student's *t* test. When comparing differences in proportions, Fisher's exact test or the χ^2 test were used, as appropriate.

Results

'EARLY ONSET' AND 'LATE ONSET' ISCHAEMIC BRAIN LESIONS (PERIVENTRICULAR LEUCOMALACIA)

Periventricular areas of increased echogenicity consistent with ischaemic brain lesions were diagnosed in 39 (17%) of 232 babies from the videotape recordings.[5] These areas of increased echogenicity were associated with or progressed to multiple periventricular cysts (periventricular leucomalacia).[3] The time of ultrasound diagnosis of such ischaemic brain lesions varied between one and 70 days. In nine (23%) of 39 babies, lesions consistent with brain ischaemia were present on the initial scan carried out within two hours of birth (range 30 minutes to 2 hours; median 30 minutes). These nine babies were in the early onset group of ischaemic brain lesions; six survived and were followed up after they had left hospital. A total of 30 babies (77%) developed lesions consistent with brain ischaemia between 4 and 70 days postnatally (median 7 days); 15 babies who survived were followed up.

ANTENATAL AND BIRTH HISTORY

Table 1 shows the antenatal and birth history in babies with early onset and late onset ischaemic brain lesions. The only significant difference between the two groups was a higher proportion of babies with a history of intrauterine growth retardation among those with early onset lesions. These babies were also 'small for dates', as their birth weights were below the 3rd centile for gestational age.[10] Mothers who had vaginal bleeding at any time during the second or third trimesters of pregnancy, or a retroplacental clot on examination of the placenta, were deemed to have had an antepartum haemorrhage (table 1). The indications for caesarean section (table 1) in the early onset group with brain ischaemia were late fetal heart rate decelerations in the cardiotocogram recordings (n=3), and intrauterine growth retardation (n=1). In the late onset group, the caesarean sections were carried out for pre-eclamptic toxaemia (n=5), antepartum haemorrhage (n=3), prolonged rupture of membranes (n=2), breech presentation (n=1), intrauterine growth retardation (n=1), and late fetal heart rate decelerations (n=7).

Table 1 Antenatal and birth history

	Ischaemic brain lesions	
	Early onset (n=9)	*Late onset (n=30)*
Antenatal conditions:		
Pre-eclamptic toxaemia	1	8
Prolonged rupture of membranes (>24 hours)	4	5
Antepartum haemorrhage	5	12
Late fetal heart rate decelerations	3	7
Intrauterine growth retardation	5	1**
Mode of delivery:		
Vaginal:		
Cephalic	5	8
Breech	0	2
Forceps	0	1
Caesarean section:		
In labour	3	10
Before labour	2	9
Birth:		
Birth asphyxia—Apgar score:		
<5 At 1 minute	2	16
<5 At 5 minutes	2	10
Mean (SD) birth weight (g)	1093 (496)	1237 (329)
Mean (SD) gestational age (weeks)	28·8 (1·8)	28·4 (2·6)
Small for dates	5	1**

**p<0·005, Fisher's exact test.

NEONATAL EVENTS

Various neonatal events that occurred in the two groups of babies are shown in table 2. There were significant differences in the frequency of hyaline membrane disease and of recurrent apnoea. A higher proportion of babies with late onset ischaemic brain lesions had hyaline membrane disease. By contrast, a greater proportion of those with early onset brain lesions had recurrent apnoea, which was severe enough for the babies to require respiratory support by mechanical ventilation. The cessation of respiration during apnoea was associated with bradycardia, the heart rate slowing typically to less than 100 beats/minute. Brain haemorrhage was associated with the ischaemic lesions in similar proportions of babies in the two groups (table 2).

DEVELOPMENTAL OUTCOME

In later childhood, four of the six surviving babies with early onset ischaemic brain lesions had disabilities—spastic diplegia, impaired vision, sensorineural hearing loss, squints, and developmental delay (table 3). None of the four

Table 2 Neonatal events

	Ischaemic brain lesions	
	Early onset (n=9)	*Late onset (n=30)*
Neonatal conditions:		
Hyaline membrane disease	3	22*
Recurrent apnoea	6	3**
Seizures (treated with anticonvulsant drugs)	4	12
Patent ductus arteriosus (treated with indomethacin)	3	9
Septicaemia (positive blood cultures)	2	7
Pneumothorax	2	12
Muscle paralysis (pancuronium)	2	15
Brain haemorrhage (ultrasound scans):		
Subependymal haemorrhage	2	3
Intraventricular haemorrhage only	4	15
Intraventricular haemorrhage with parenchymal extension	0	4
Blood gas analysis (first four days of birth):		
Arterial oxygen pressure <6·7 kPa in >60% oxygen	6	26
Arterial carbon dioxide pressure >8·0 kPa	1	15
pH <7·2 with base deficit >10·0	1	24
Death	3	15

*p<0·05, **p<0·005, Fisher's exact test.

Table 3 Outcome in later childhood (18 to 48 months) in survivors with ischaemic brain lesions

Clinical findings	*Ischaemic brain lesions*	
	Early onset (n=6)	*Late onset (n=14)*
Spastic diplegia	3	5
Impaired vision	1	3
Squints	3	4
Sensorineural deafness	1	1
Developmental delay	4	3
No of children with any one of above disabilities	4	7
No of children with no detectable abnormality	2	7

children with disabilities had a history of late decelerations of fetal heart rate on cardiotocographic tracings, or of birth asphyxia (Apgar score <5 at 1 minute). One of the remaining two children with no disability had a history of birth asphyxia. In the 15 babies with late onset ischaemic brain lesions, one died during the first three months after leaving hospital of sudden infant death syndrome. Of the remaining 14 children, seven had disabilities similar to those in the 'early onset' group. Five of the seven children with disabilities had histories of late fetal heart rate decelerations or birth asphyxia. In the remaining seven children with no disabilities, two had similar histories of birth asphyxia. In these 11 children with disabilities in later childhood, ischaemic lesions of early onset had contributed to brain damage in a third of the total number of cases.

Discussion

The risks of periventricular leucomalacia of early onset seems to be increased in babies with a history of intrauterine growth retardation. Periventricular leucomalacia is caused by infarction in areas of the brain that are especially vulnerable to reduced perfusion, including the 'watershed' areas distributed between the 'ventriculofugal' and 'ventriculopetal' branches of cerebral blood vessels.[11] It is also possible that these areas become damaged when hypoxic episodes give rise to cerebral oedema, as an increase in intracranial pressure as a result of oedema may reduce cerebral perfusion. Intrauterine growth retardation has been associated with certain conditions including impaired uteroplacental blood flow[12] and a reduction in cardiac glycogen reserves.[13] The former might be expected to increase the likelihood of fetal hypoxia, while the latter may lead to a progressive reduction in cardiac output when stressed by hypoxia and thus limit the ability of the growth retarded fetus to maintain its blood pressure. A similar sequence of events may have resulted in a reduction in cerebral blood flow in our infants with a periventricular leucomalacia of early onset.

We cannot be precise about the timing of ischaemic brain lesions diagnosed soon after birth from the obstetric information available. In some babies who had no history of late fetal heart rate deceleration, or low Apgar scores (<5 at 5 minutes) associated with the ultrasound appearance of brain ischaemia, the brain damage may have taken place before birth. In others who did have histories of fetal hypoxia or birth asphyxia, these conditions may have predisposed to ischaemic brain lesions that progressed postnatally. It is possible that blood pressure recordings carried out shortly after birth (unpublished observations) may improve the recognition of babies who are poorly perfused. Treatment aimed at the maintenance of blood pressure, which includes giving plasma and inotropic drugs, may improve cerebral blood flow and thus lead to a decrease in the incidence of periventricular leucomalacia.

Recurrent apnoeic episodes and bradycardia may have a role in exacerbating hypoxic-ischaemic brain damage in preterm babies.[14] Such apnoeic episodes may lead to a reduction in systemic blood pressure, and a reduction in cerebral blood flow, depending on the severity of the decline in heart rate (bradycardia).[14] Our ultrasonographic findings suggest that the ischaemic insult to the brain in the early onset group had preceded the apnoeic episodes. In this setting, repeated episodes of apnoea and bradycardia may potentiate the progress of ischaemic injury to periventricular leucomalacia in the arterial border zones that are most susceptible to damage. Ventilating such babies could have the desired effect of preventing bradycardia associated with apnoeic episodes. The subsequent progress of these babies may, however, be complicated by periventricular haemorrhage from blood vessels that had previously been damaged by ischaemia.

Ischaemic brain damage of late onset seems to have a different aetiology, as we found an association with hyaline membrane disease in babies who were appropriately grown for their gestational ages. The relationship between hyaline membrane disease and periventricular haemorrhage is well known.[11] In similar babies, serial brain scans carried out by us showed ultrasound appearances consistent with periventricular leucomalacia after the development of periventricular haemorrhage.[5] Though the reasons for this are not clear it may be that a reduction in carbon dioxide pressure during mechanical ventilation,[2] a fall in systemic blood pressure from blood loss caused by the haemorrhage, or an intracerebral haematoma[15] may have contributed to a reduction in regional cerebral blood flow and ischaemic brain damage.

By only including babies who developed cystic brain lesions (periventricular leucomalacia) in our study, we are confident in the selection of those with ischaemic brain damage. Unlike periventricular haemorrhage, which does not usually occur after the first seven days of life, periventricular leucomalacia in our study occurred over a wider time period. This, together with the progression of areas of increased echogenicity in the periventricular region to cystic lesions in ultrasound brain scans, is consistent with previous reports.[3 16 17] We are aware that having excluded babies in whom areas of echogenicity did not progress to cystic lesions it is possible that some babies with ischaemic brain damage were not considered in our study, as cystic lesions less than 3 mm in size are not likely to be recognised by the present scanning method.[3 16] In cases similarly selected we have previously suggested that the high neonatal mortality rate and the development of ventricular enlargement as a result of brain atrophy are the immediate consequences of ischaemic brain damage. In the present study the late complications of such damage appear to be cerebral palsy, sensorineural hearing loss, and visual impairment.

In a third of the babies in this study with disabilities (including cerebral palsy in later childhood) the ischaemic brain damage may have already taken place by the time of birth. This has implications for our understanding of mechanisms underlying abnormal development in preterm babies and emphasises the greater risks attached to intrauterine growth retardation. Obstetric management directed towards optimal timing and mode of delivery should seek to prevent the early onset of periventricular leucomalacia.

Financial support from the North Western Regional Health Authority is gratefully acknowledged.

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Prediction of acute renal failure after birth asphyxia

D S Roberts, G B Haycock, R N Dalton, C Turner, P Tomlinson, L Stimmler, J W Scopes

Abstract

Twenty one babies of 34–41 weeks' gestational age with birth asphyxia (5 minute Apgar score $\leqslant 5$ or umbilical artery pH $\leqslant 7{\cdot}2$) were studied during the first two days of life to find out whether the urinary excretion of tubular markers of renal function is of value in the early diagnosis of acute renal failure. Urinary retinol binding protein, myoglobin, and N-acetyl-β-D-glucosaminidase (NAG), expressed as a ratio with urinary creatinine, were measured and excretion profiles repeated at 3–6 days in 15 infants and at 7–14 days in 11 infants. Plasma creatinine concentration, creatinine clearance, plasma myoglobin concentration, and fractional sodium excretion were measured where possible in asphyxiated infants. Control data were obtained from 50 healthy infants: 28 gave urine samples alone, 17 urine and blood, and five blood alone. Normal urinary values were derived from 17, 25, and three infants, respectively, for the three time periods. The number of control samples was limited for ethical reasons.

Four asphyxiated infants had acute renal failure (group 1), four had tubular dysfunction without glomerular disturbance (group 2) and 13 had normal renal function (group 3). Group 1 were clearly identified by greatly increased urinary retinol binding protein (>27 000 μg/mmol creatinine) and myoglobin (>1500 μg/mmol creatinine) excretion measured in the first two days of life. In control infants the range of excretion of retinol binding protein within the same time period was 3 to 967 μg/mmol creatinine and urinary myoglobin was undetectable. Excretion of NAG failed to discriminate between groups 1 and 2. Acute renal failure occurred only in infants who had heavy myoglobinaemia. Tubular dysfunction in group 2 was transient and not accompanied by plasma electrolyte disturbances.

We conclude that measurement of urinary excretion of retinol binding protein or myoglobin after birth is helpful in the early diagnosis of acute renal failure.

Acute renal failure is a recognised complication of birth asphyxia; it carries a poor immediate prognosis and may result in permanent renal damage in up to 40% of survivors.[1–6] The recent reports of increased urinary excretion of β_2-microglobulin in infants with evidence of intrauterine fetal distress (meconium stained liquor) by Cole *et al*, and in sick infants by Tack *et al*, led these authors to suggest that subclinical, hypoxic, renal damage may be a relatively common, underdiagnosed finding.[7 8]

The early recognition of acute renal failure is particularly important in asphyxiated infants with hypoxic encephalopathy, in whom a stable biochemical milieu is vital, because it facilitates the administration of appropriate fluid and electrolyte replacement. The diagnosis is frequently difficult to make, however, because many of the clinical and biochemical findings that are helpful in establishing the diagnosis of acute renal failure in adults and children are unreliable in neonates. The presence or absence of oliguria may be misleading as 7% of healthy babies fail to pass urine until the second day of life,[9] and non-oliguric acute renal failure has been reported in neonates. The plasma creatinine concentration on day 1 is a poor guide to an infant's renal function, because it mainly reflects the maternal creatinine concentration.[11 12] A further difficulty in the interpretation of the plasma creatinine concentration is that the methods of measurement in common use (modifications of the colorimetric Jaffé reaction) are subject to errors from interfering chromogens such as bilirubin and pyruvate,[13] substances that are likely to be present in increased concentrations in the blood of sick infants. The value of indexes such as the urine:plasma concentration ratio of sodium, creatinine, and urea are limited by the overlap that exists between infants with functional (prerenal) failure, and those with intrinsic renal failure.[14–16] The fractional excretion of sodium or renal failure index may be of diagnostic value, but both need to be interpreted with caution in the preterm,[17] and in infants who have been given a saline challenge or treated with diuretics or aminophylline.

Myoglobinuria is a cause of renal failure in adults,[18] and a strong correlation between the duration of postnatal oliguria and the severity of myoglobinaemia has been reported in asphyxiated infants.[19] The presence of myoglobinuria may therefore be a useful indicator of acute renal failure.[20]

This study was designed to investigate the value of measurements of the excretion of retinol binding protein, myoglobin, and N-acetyl-β-D-glucosaminidase (NAG) during the first two days of life as predictors of clinically important acute renal failure after birth asphyxia, and to follow the excretion profiles into the second week of life.

Patients and methods

Twenty one infants who had had an episode of birth asphyxia, defined as a 5 minute Apgar

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score of 5 or below, or an umbilical artery pH of 7·2 or below at delivery, were studied in the neonatal units of these hospitals. The study was approved by the ethics committees of both hospitals and parental consent was obtained in all cases. Infants were managed according to the standard protocols used in the two units, no modifications being made as a result of participation in the study.

Causes of birth asphyxia included severe shoulder dystocia, intrapartum cord traction, and haemorrhage. Sepsis was implicated in the cause in only one infant, delivered in poor condition at 36 weeks, whose cultures yielded *Listeria monocytogenes* after 48 hours' incubation.

All infants were of 34 weeks' gestational age or above. This lower limit was chosen because nephronogenesis is complete by this time and comparisons are not usually complicated by developmentally determined differences in renal function.[21] Gestational age was assessed from the menstrual history, ultrasonography, or by the clinical criteria of Dubowitz *et al*.[22]

Wherever possible, infants were studied on several occasions during the first two weeks of life and results grouped into three postnatal time periods: 1–2 days, 3–6 days, and 7–14 days. All infants were studied within the first two days of life and measurements were repeated between 3–6 days in 15, and in the second week of life in 11 infants. It was not possible to repeat measurements in some infants because of death or discharge.

Table 1 Number of infants in each group providing samples for each analysis at each time period

Analysis	*Asphyxiated infants (n=21)*			*Control infants* (n=50)*		
	Time period (days)			*Time period (days)*		
	1–2	*3–6*	*7–14*	*1–2*	*3–6*	*7–14*
Retinol binding protein	21	15	11	17	27	3
Urinary myoglobin	21	15	11	17	27	3
N-acetyl-β-D-glucosaminidase	21	15	11	17	27	3
Urine output	19	0	0	0	0	0
Plasma creatinine	21	14	10	4	13	3
Creatinine clearance	19	14	9	0	0	0
Plasma myoglobulin	20	14	8	4	14	3
Fractional excretion of sodium	19	14	9	4	11	2

*Of the 50 control infants, 28 gave urine samples, five gave blood samples, and 17 gave both.

Table 2 Clinical data of infants studied on days 1–2. Values are expressed as geometric mean (range)

	Asphyxiated infants (n=21)	*Control infants (days 1–2) (n=17)*	*p Value**
Gestational age (weeks)	39·3 (34–41)	37·7 (34–41)	NS
Birth weight (g)	3400 (2200–4800)	3400 (1900–4700)	NS
Apgar score:			
At 1 minute	2·3 (0–7)	8·6 (6–10)	<0·01
At 5 minutes	4·1 (0–9)	9·6 (8–10)	<0·01

*Wilcoxon rank sum test with Bonferroni correction.

A total of 50 non-asphyxiated infants served as controls for the study; 28 provided urine samples, 17 both urine and blood samples, and five urine samples alone. Seventeen control urine specimens were available for analysis during the first two days of life, 27 during the 3–6 day period, and three during the period 7–14 days. With the exception of two infants who provided urine samples on different days, non-asphyxiated infants were studied on a single occasion.

Blood samples from all infants were obtained when blood was drawn for clinical purposes, and data are limited for this reason. The numbers of individual analyses are shown in table 1. Plasma was separated immediately and stored at −20°C until analysis. Urine was collected by the method of Tarlow and also stored at −20°C.[23] None of the infants studied was treated with aminoglycosides, which may affect tubular function.[24 25]

Glomerular function was estimated by the plasma creatinine concentration and endogenous creatinine clearance (calculated as urine creatinine concentration×urine flow rate (ml/min)/ plasma creatinine concentration). Tubular performance was assessed by the urinary concentrations of retinol binding protein, NAG, and myoglobin, divided by the urinary creatinine concentration. Fractional excretion of sodium (calculated as the urine:plasma sodium ratio/ urine:plasma creatinine ratio) was also measured, and results are reported when diuretics were not administered.

Sodium was estimated in plasma and urine by flame photometry. Creatinine was measured in plasma by high performance liquid chromatography with cation exchange (HPLC),[26] and in urine by an automated, reaction rate version of the Jaffé reaction.[27] Retinol binding protein and myoglobin were measured in urine by enzyme linked immunosorbent assays (ELISA) using rabbit antisera (Dako Ltd).[28] (Retinol binding protein ELISA: P Tomlinson, personal communication, myoglobin ELISA: D Roberts, unpublished observations). Myoglobin in plasma was also estimated by ELISA. The limit of detection for both assays was 2 μg/l. NAG

Table 3 Results of biochemical tests done during first two days of life. Values expressed as geometric mean (range)

	Control infants (n=17)	*Asphyxiated infants*		
		Group 1 (n=4)	*Group 2 (n=4)*	*Group 3 (n=13)*
Urinary excretion of:				
Retinol binding protein (μg/mmol creatinine)	75 (3–967)	42 906 (27 759–81 112)	7570 (3721–15 243)	154 (6–1418)
N-acetyl-β-D-glucosaminidase (μmol substrate/hour/mmol creatinine)	134 (32–362)	4707 (1135–13 484)	1520 (692–7736)	153 (24–310)
Myoglobin (μg/mmol creatinine)	Not detectable	6004 (1526–13 195)	4 (0·4–430)	1 (Not detectable–17)
Fractional sodium excretion (%)	0·2 (0·1–0·5)	32 (13–71)	1·7 (0·9–3·4)	0·3 (0·1–2·0)
Creatinine clearance (ml/min/kg)	Not measured	0·01 (0·003–0·04)	0·8 (0·6–1·0)	1·2 (0·4–3·1)
Plasma concentrations of:				
Creatinine (μmol/l)	75 (59–93)	257 (188–344)	101 (77–116)	78 (51–116)
Myoglobin (μg/l)	48 (32–73)	2071 (855–7625)	104 (38–262)	95 (21–390)
Urine output (ml/kg/hour)	Not measured	0·3 (0·1–0·8)	2·1 (1·5–2·7)	2·0 (0·5–5·7)

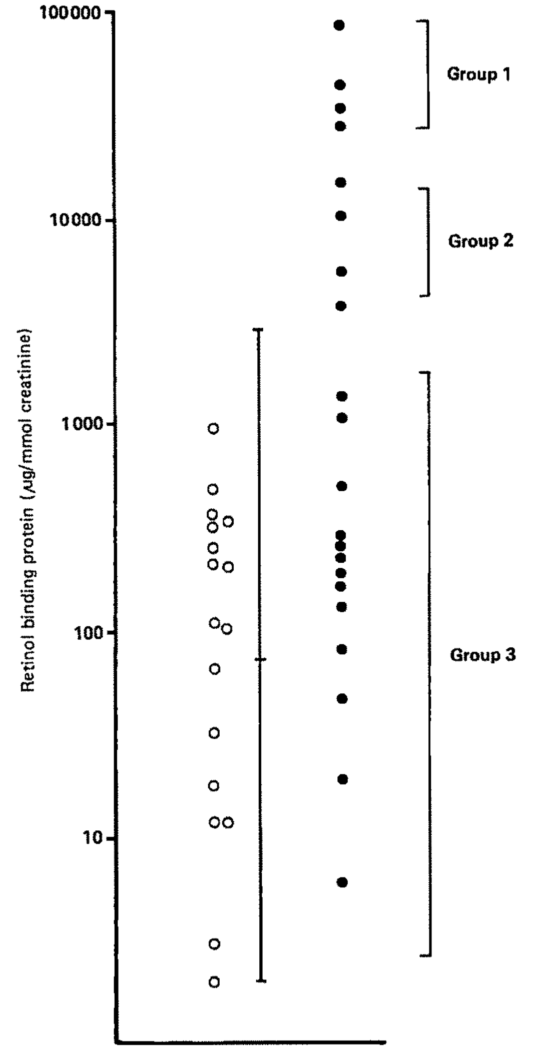

Figure 1 Urinary concentration of retinol binding protein measured during the first two days of life in control (open symbols) and asphyxiated infants (closed symbols). Asphyxiated infants were divided into three subgroups according to the degree of retinol binding protein excretion. The scale is logarithmic, and the bar indicates the mean and tolerance limits of normal.

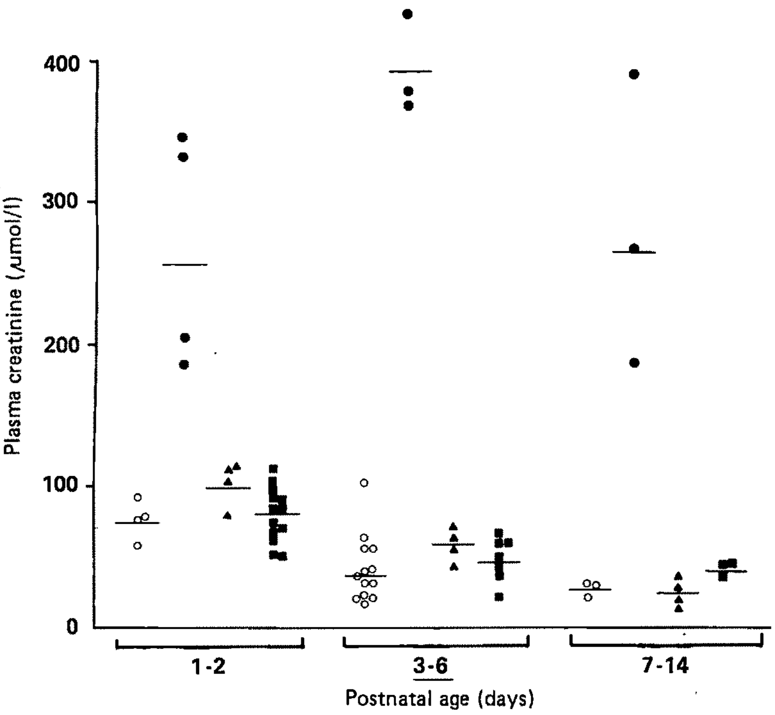

Figure 2 Plasma creatinine concentrations with increasing postnatal age in control subjects (open symbols) and the three subgroups of asphyxiated infants (closed symbols—group 1: circles, group 2: triangles, and group 3: squares). The horizontal bars indicate the mean for each group.

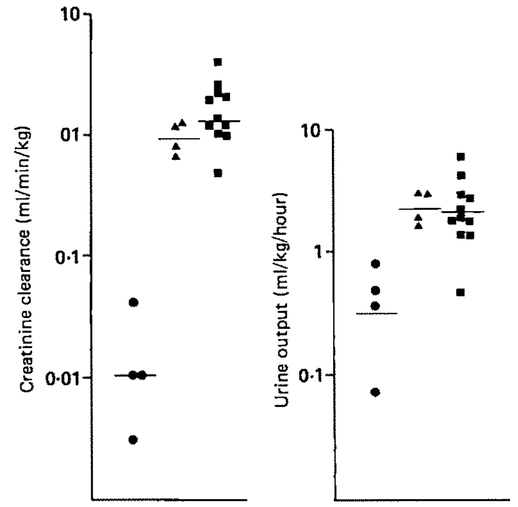

Figure 3 Creatinine clearance according to birth weight, and urine output, measured during the first two days of life in the three subgroups of asphyxiated infants (group 1: circles, group 2: triangles, and group 3: squares). The scale is logarithmic, and the horizontal bars indicate the mean for each group.

activity was measured by an automated colorimetric method using p-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate (Sigma Chemical Co).[29]

When several estimations were made in any infant for each study period, the arithmetic mean was calculated and used in further analyses.

Grouped results are presented in the figures as individual data points and in the tables as geometric means and ranges.

Results

Table 2 shows the clinical data of the study infants on days 1–2. There were no significant differences in gestational age or birth weight between the asphyxiated infants and controls.

Four asphyxiated infants (19%) developed acute renal failure (defined as a plasma creatinine concentration of over 130 μmol/l for at least two consecutive days). One infant required treatment with peritoneal dialysis for hyperkalaemic dysrhythmia.

Nine infants (43%) had asphyxial encephalopathy with seizures and seven of these had some degree of renal dysfunction (three had acute renal failure, and four had tubular proteinuria). A further six infants (29%) had transient cerebral irritability and feeding problems, but only one of these infants developed acute renal failure. Six infants who had no cerebral symptoms had normal renal function. Three infants died (14%), two during the course of the study, at 2 and 10 days of age, respectively.

The relevant biochemical data of the study infants on days 1–2 are shown in table 3. The upper tolerance limits of normal for retinol binding protein and NAG were defined as the geometric mean plus two logarithmic SDs for the respective set of control data. These were 2968 μg/mmol creatinine and 465 μmol substrate/hour/mmol creatinine, respectively. Myoglobin was undetectable in the urine of healthy newborns on days 1–2.

When the urinary retinol binding protein:

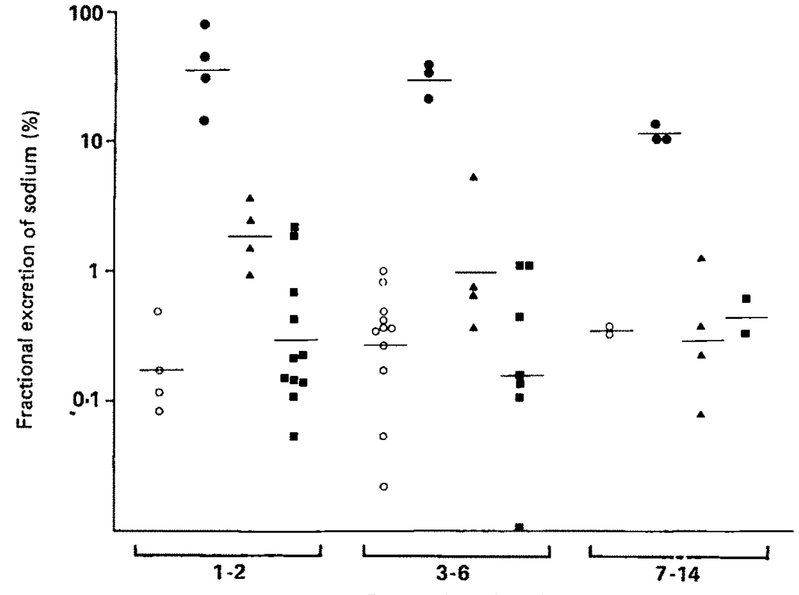

Figure 4 Fractional excretion of sodium with increasing postnatal age in control subjects (open symbols) and asphyxiated infants (closed symbols—group 1: circles, group 2: triangles, and group 3: squares). The scale is logarithmic and the horizontal bars indicate the mean for each group.

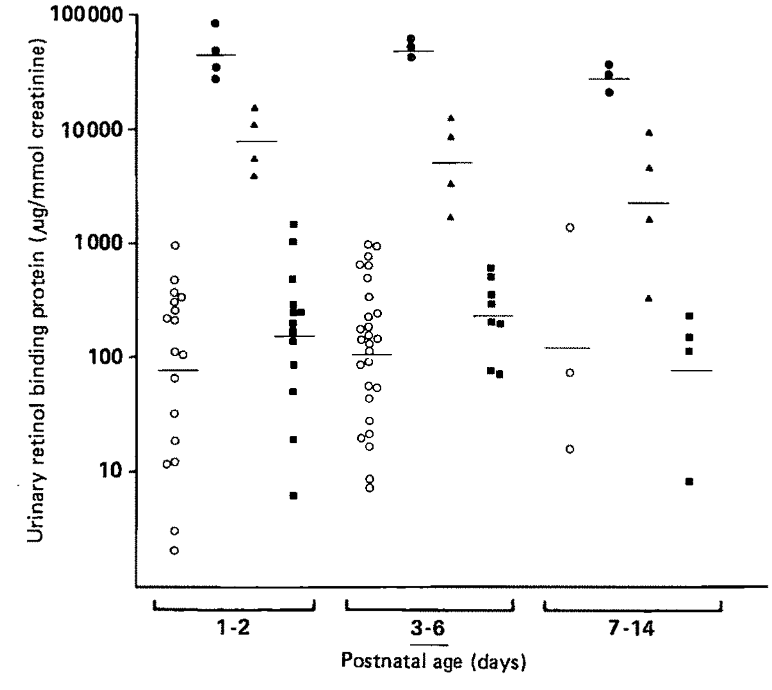

Figure 5 Urinary excretion of retinol binding protein with increasing postnatal age in control subjects (open symbols) and asphyxiated infants (closed symbols—group 1: circles, group 2: triangles, and group 3: squares). The scale is logarithmic and the horizontal bars indicate the mean for each group.

creatinine ratio of asphyxiated infants (measured in the first two days of life) was displayed graphically, the values were noted to be divided into three distinct subgroups (fig 1). Group 1 (n=4) had concentrations that were 15–44 times above the upper tolerance limit of normal. Group 2 (n=4) had concentrations that were 2–8 times above the upper tolerance limit of normal, and group 3 (n=13) had concentrations that were within the normal range.

All infants in group 1 had acute renal failure. Glomerular function was normal in groups 2 and 3. Throughout the rest of this paper the asphyxiated infants are considered in these three subgroups.

Plasma creatinine values in control infants and in the asphyxiated infants studied serially are shown in fig 2. Infants in group 1 already had greatly increased plasma creatinine concentrations during the first two days of life, which increased further towards the end of the first week and then declined during the second week. Infants in groups 2 and 3 showed the expected decline in plasma creatinine concentration with increasing postnatal age.

Creatinine clearance during the first two days of life, expressed according to birth weight, was correspondingly reduced in group 1; all these infants were oliguric at the time of initial assessment with urinary flow rates of less than 1 ml/kg/hour (fig 3).

Tubular dysfunction was confirmed in groups 1 and 2 by the fractional excretion of sodium (fig 4).

In control infants the mean urinary excretion of retinol binding protein and myoglobin, and the fractional excretion of sodium were constant during the first two weeks of life; NAG excretion showed a slight rise with increasing postnatal age. Myoglobin was detected in the urine of only one control infant, between 3 and 6 days: the concentration was at the limit of the assay and the infant did not show the highest concentration of retinol binding protein or of NAG in the control group. In infants in group 1 the mean excretion of all markers declined towards their respective normal ranges with increasing time after the episode of asphyxia, the retinol binding protein most slowly. The same was true of group 2 with the exception of NAG, the mean excretion of which remained unchanged. Excretion of all markers in group 3 was comparable with control values throughout the first two weeks of life (fig 5, 6, and 7).

Free myoglobin seems to be handled by the kidney as a low molecular weight protein, in a similar way to retinol binding protein. In the presence of an increased filtered load (a raised plasma concentration) it appears in the urine in detectable quantities in the presence of tubular dysfunction, as indicated by an increased urinary retinol binding protein concentration.

Plasma myoglobin concentration was greatly increased in infants in group 1 (fig 8), but concentrations decreased in all groups with increasing postnatal age.

Discussion

The measurement of various tubular markers of renal function has proved useful in the diagnosis and surveillance of a number of renal disorders.[30–33] Low molecular weight proteins, such as β_2-microglobulin and retinol binding protein, are freely filtered at the glomerulus and almost completely reabsorbed by the proximal tubular cells in health, so that when tubular dysfunction is present, increased quantities appear in the urine. The report of raised urinary β_2-microglobulin concentrations in infants with meconium stained liquor who were otherwise well led to the suggestion that subclinical tubular damage was present in a proportion of these infants, and that measurement of low

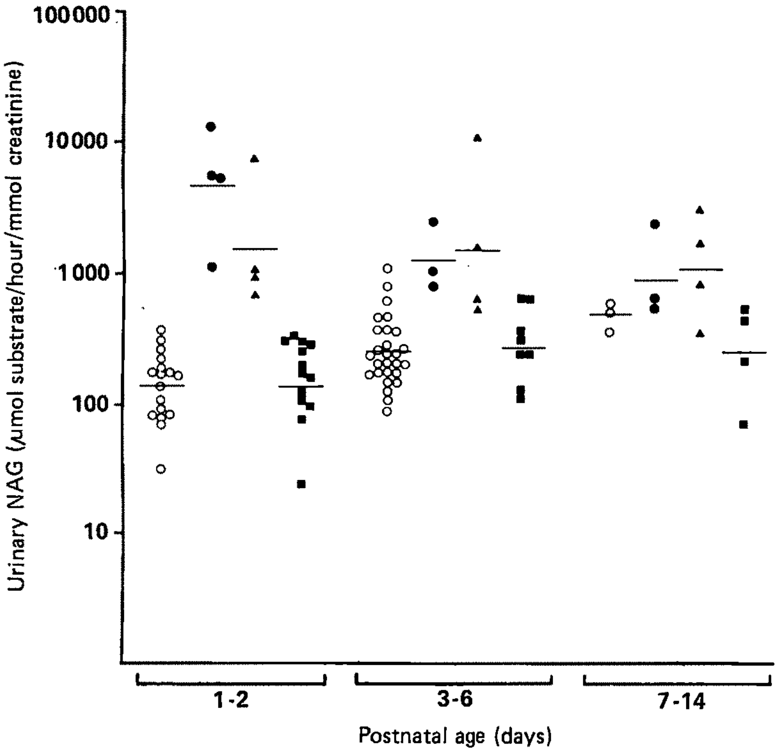

Figure 6 Urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) with increasing postnatal age in control subjects (open symbols) and asphyxiated infants (closed symbols—group 1: circles, group 2: triangles, and group 3: squares). The scale is logarithmic and the horizontal bars indicate the mean for each group.

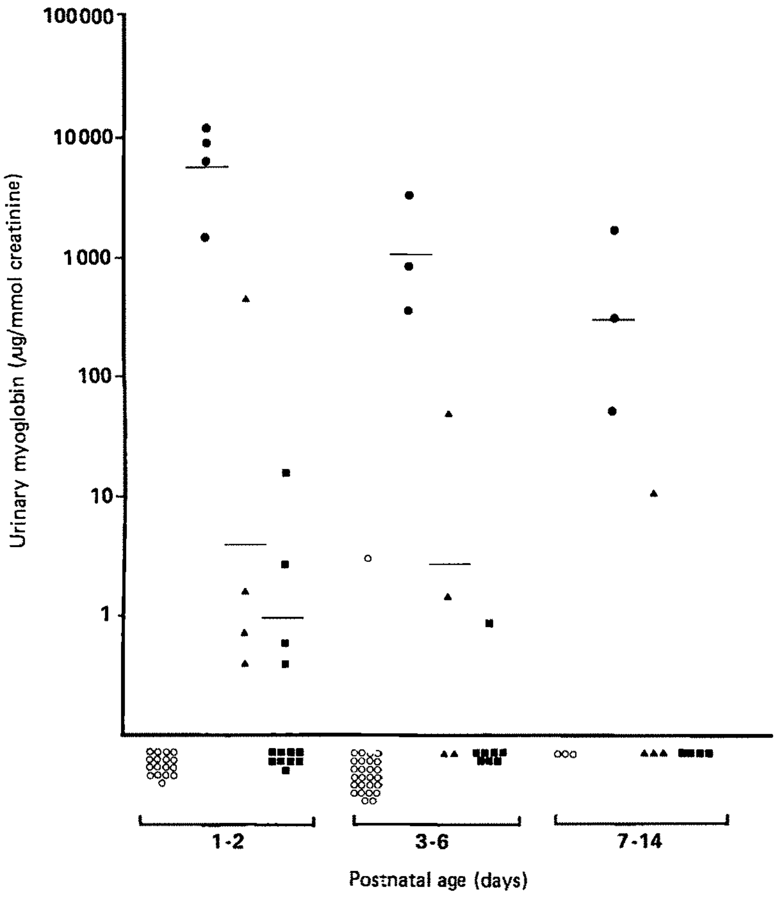

Figure 7 Urinary excretion of myoglobin with increasing postnatal age in control subjects (open symbols) and asphyxiated infants (closed symbols—group 1: circles, group 2: triangles, and group 3: squares). The scale is logarithmic and the horizontal bars indicate the mean for each group.

molecular proteinuria might be a useful adjunct in the assessment of their renal function.[7]

Comparison data from studies in adults have shown that urinary retinol binding protein concentration correlates well with that of β_2-microglobulin, and that it is of equal sensitivity in the detection of tubular dysfunction.[34] We chose to measure retinol binding protein because it is stable at lower urinary pH values at which degradation of β_2-microglobulin occurs, and the need for manipulation of urinary pH on voided specimens is eliminated.[35]

Excretion of retinol binding protein in neonates is higher than in adults. Our control infants achieved a mean retinol binding protein excretion in the first two days of life of 75 µg/mmol creatinine, with similar values in the second week of life, in contrast to a reported mean concentration of 7·7 µg/mmol creatinine in adult men.[28] This tenfold difference is not a spurious increase in the retinol binding protein concentration as a result of the lower urinary creatinine concentrations found in infants; the absolute urinary retinol binding protein concentrations in the infants were greater, with a maximal recorded value of 4000 µg/l, compared with the maximal normal adult value reported in the above study of 540 µg/l. This presumably reflects reduced proximal tubular reabsorption in neonates.

The mean retinol binding protein excretion reported here for control infants with gestational ages of more than 34 weeks is similar to the value obtained by Clark *et al*,[36] but out upper tolerance limit of normal is roughly five times higher and reflects a greater range of retinol binding protein excretion values in the group studied.

We identified three groups of asphyxiated infants according to their urinary retinol binding protein:creatinine ratio. The four infants with acute renal failure had the highest excretion (range 27 759–81 112 µg/mmol creatinine) and there was clear discrimination between these infants and the others. In turn, infants in group 2 (3721–15 243) were clearly differentiated from those in group 3 (6–1418). Infants with acute renal failure also had the heaviest myoglobinuria (range 1526–13 195 µg/mmol creatinine) and, as with retinol binding protein, were sharply distinguished from the others. Those in group 2 had detectable, but lesser, degrees of myoglobinuria (0·4–430). Gross pigmenturia, giving a brown colour to the urine, was seen in all infants in group 1, and in the infant from group 2 who had the highest myoglobin concentration of that group. In three infants from group 3 myoglobin was detected in the urine, but the values were at the limit of detection. Our results are in agreement with those of Kojima *et al.*[20] Tubular dysfunction was confirmed by the fractional excretion of sodium, which was grossly abnormal in group 1 (mean 31·9%) mildly increased in group 2 (1·7%), and normal in group 3 (0·3%). That in control infants was below 1% and in sodium balance studies the fractional excretion of sodium in healthy infants of similar gestational age with a sodium intake of ≤2·5 mmol/kg/day has been reported to be normally less than 1%.[17]

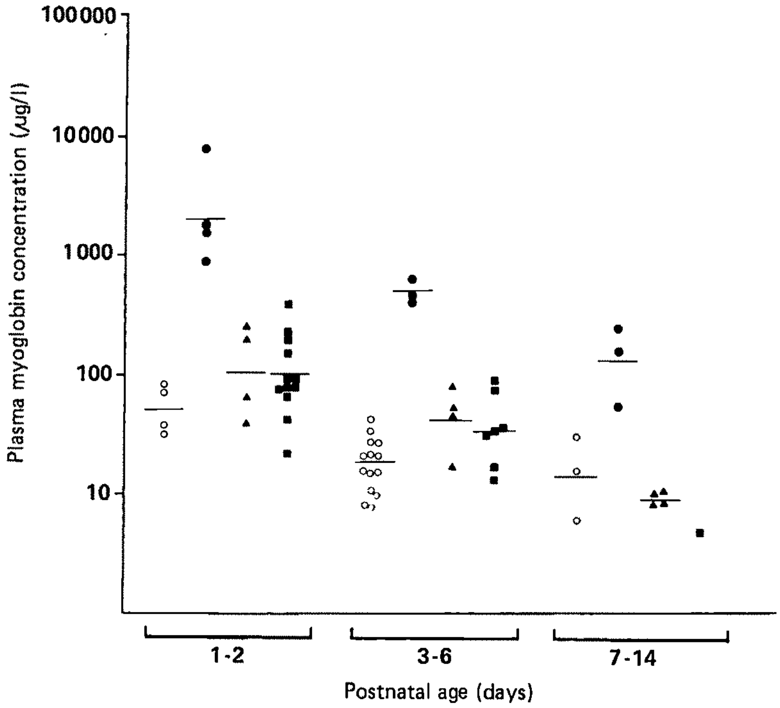

Figure 8 Plasma myoglobin concentrations with increasing postnatal age in control subjects (open symbols) and asphyxiated infants (closed symbols—group 1: circles, group 2: triangles, and group 3: squares). The scale is logarithmic and the horizontal bars indicate the mean for each group.

NAG is a lysosomal enzyme of a molecular weight that precludes its filtration at the glomerulus, it is also rapidly cleared from the circulation by the liver so that increased urinary excretion is a consequence of intrinsic renal damage.[37] NAG excretion was considerably increased in infants with acute renal failure (range 1135–13 484 μmol substrate/hour/mmol creatinine) and in infants with tubular dysfunction and no glomerular disturbance (692–7736). This presumably reflects a similar initial degree of tubular cell damage in the two groups. NAG is clearly a sensitive measure of tubular damage, but not specific enough to discriminate the infants with acute renal failure.

In the infants who were studied serially, retinol binding protein, NAG, and myoglobin excretion declined in group 1, retinol binding protein more slowly than either NAG or myoglobin, and all remained above the normal range into the second week of life. In one infant, measurements were repeated at the age of six weeks and urinary retinol binding protein and NAG were still greatly increased, suggesting that tubular recovery, if it occurs at all, is slow. The increased excretion of NAG in the second week of life is probably not the result of continued tubular cell exfoliation, but of increased lysosomal activity in surviving cells; tubular reabsorption of low molecular weight proteins occurs by endocytosis, the proteins then fusing with lysosomes within which they are digested. Urinary NAG concentrations were highest in those infants with the greatest excretion of retinol binding protein and myoglobin, suggesting that this persistent use is the result of a stimulated exocytosis similar to that shown in animal experiments.[38] The similar continued rise in urinary NAG, with more or less constant mean values throughout the two week postnatal period in infants in group 2, supports this suggestion. In these infants retinol binding protein and myoglobin excretion steadily declined, and by the second week remained high in only one infant; measurements were repeated in the third week in this infant and all results were then normal. This indicates that tubular dysfunction in these infants is transient. Infants in group 3 had concentrations comparable with those in control infants throughout the study.

Our single criterion for the diagnosis of acute renal failure was a plasma creatinine concentration of over 130 μmol/l for at least two days. The presence of oliguria was not required so we did not overlook infants with non-oliguric acute renal failure. Similar plasma creatinine concentrations have been used as diagnostic criteria in other studies.[1 2 39] We measured plasma creatinine using high performance liquid chromatography because the method is specific; with modified Jaffé techniques various chromogens present in plasma interfere with the reaction resulting in overestimations or underestimations of the creatinine concentration that cannot be predicted for any individual measurement and may produce errors in excess of 100%.[40] Within two days, the plasma creatinine concentrations in infants in group 1 were considerably raised (mean 257 μmol/l). The mean values were similar in groups 2 and 3, comparable to our limited control data, and within the expected range for infants of similar postnatal age reported by other workers.[11 12] Creatinine clearance expressed according to birth weight,[41] was correspondingly reduced (mean 0·01 ml/min/kg) in group 1. The clearances measured in groups 2 (0·8), and 3 (1·2), are similar to those described by Coulthard in a review of published data on glomerular filtration rates expressed by birth weight in healthy newborns.[42]

All infants in group 1 were oliguric in the first two days of life with urinary flow rates of less than 1 ml/kg/hour. One infant from group 3 had a reduced urine output, the remainder had flow rates in excess of 1 ml/kg/hour.

Severe muscle damage associated with subsequent renal failure is well documented in adults,[18] and the same phenomenon probably occurs in neonates.[43] Birth asphyxia is associated with myoglobinaemia in the first few days of life, the severity of which is—in turn—related to the duration of postnatal oliguria.[19] Plasma myoglobin concentrations, measured in the first 2 days of life in our asphyxiated infants, ranged from 21 to 7625 μg/l. The infants with acute renal failure had the highest concentrations, and the infant who required dialysis had the highest of all. These concentrations are similar to those reported by Kasik *et al*,[19] and similar to those seen in adults with acute rhabdomyolysis and acute renal failure.[44] At birth, infant muscle contains roughly 10% of the adult myoglobin content[45]; the plasma concentrations achieved by several of the asphyxiated infants therefore indicate considerable muscle breakdown.

If the normal range of plasma creatinine at birth and an expected daily rise above 18 μmol/l in the presence of acute renal failure are considered,[46] the plasma creatinine concentrations in our infants represent an extremely rapid rise. Such a rise has been reported in myoglobinuric renal failure in adults, and is the result of the release of large quantities of intracellular creatine from damaged muscle.[47] Our results show that an asphyxial insult may be associated with tubular dysfunction, but that acute renal failure supervenes only in the presence of high plasma concentrations of myoglobin: the exaggerated rise in plasma creatinine in our infants, coupled with the heavy myoglobinaemia and myoglobinuria, suggests that these infants had rhabdomyolysis induced renal failure.

Our identification of infants with no glomerular disturbance but with detectable tubular dysfunction confirms previous reports, although this was a less common occurrence in our study (19%) than in others.[7, 8] Plasma myoglobin concentrations in these infants were lower than in those with acute renal failure, evidence that they sustained less severe asphyxial damage. These infants undoubtedly have a degree of tubular dysfunction that is clinically unsuspected but which can be shown with sensitive tests. The higher mean fractional excretion of sodium in this group of infants has implications for electrolyte balance, although we found no significant differences in the plasma sodium and potassium concentrations between these infants and those without tubular dysfunction during the time periods 1–2 and 3–6 days (Wilcoxon rank sum test with correction factor).

In conclusion, 62% of the infants studied, who were over 34 weeks' gestational age and had had an episode of birth asphyxia, showed no apparent impairment of renal function, 19% had overt acute renal failure, and a further 19% had tubular dysfunction indicated by an increased excretion of tubular proteins, but without any associated glomerular disturbance. These figures do not represent the absolute incidence of renal damage in our units because the numbers presented here include infants not born in the hospital and exclude those who received treatment with aminoglycoside antibiotics.

Infants with acute renal failure were clearly identified by their increased excretion of retinol binding protein and myoglobin. Urinary retinol binding protein:creatinine ratios in excess of 27 000 μg/mmol creatinine, and urinary myoglobin:creatinine in excess of 1500 μg/mmol creatinine seem to be of diagnostic value. NAG excretion, although a sensitive indicator of tubular injury, is not specific for acute renal failure.

Plasma creatinine concentration, creatinine clearance, and fractional excretion of sodium defined the group with acute renal failure, but all have some disadvantage that affects their potential diagnostic value. The HPLC method we used, which measures 'true' plasma creatinine is of restricted availability; creatinine clearance is subject to the added inaccuracies incumbent upon timed urine collections and incomplete bladder emptying,[48] and the measurement of the fractional excretion of sodium requires simultaneous blood and urine samples and cautious interpretation when diuretics or a saline load have been administered.

The measurement of retinol binding protein or myoglobin is simple, inexpensive, and can be carried out on random samples of urine. We suggest that the measurement of retinol binding protein or myoglobin should be incorporated into the routine assessment of asphyxiated infants at birth, as they give an early indication of the adequacy of renal function.

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Area of lateral ventricles measured on cranial ultrasonography in preterm infants: reference range

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Abstract

Serial ultrasound imaging of the brain was carried out in 87 preterm infants of 27 to 36 weeks' gestational age while they were in the special care nursery and during their first postnatal year. None had evidence of intracranial disease during the neonatal period and 94% had good neurodevelopmental outcome at 2 years of age. Lateral ventricle area measurements were plotted against independent variables including postnatal age and head circumference at the time of the examination. The measurements increased as age and head circumference increased. During the first six weeks of life the mean (SD) postnatal head circumference growth velocity (rate of growth) was 0·53 (0·13) cm/week and the mean postnatal ventricular area growth velocity was 0·39 (0·19) mm^2/week.

A reference range for lateral ventricle area values was developed from these serial measurements in infants with documented good short term developmental outcome.

Real time ultrasonography is now used extensively for the detection of cerebral injuries during the neonatal period. There have, however, been few ultrasound studies of the normal size of the lateral ventricles in preterm infants.[1]

In this longitudinal study of cranial ultrasound scans, we report direct measurements of the lateral ventricular body measured by the ventricle area. This study was carried out during the first year of life in neurologically normal preterm infants. The purpose of this report is to provide reference values that can be used in the evaluation of these neonates.

Patients and methods

Between October 1984 and July 1986, preterm infants of 27–36 weeks' gestational age, who survived for the duration of the study were followed up prospectively in a longitudinal neurosonographic and clinical study. These infants were of normal weight for gestational age; Apgar score was 7 or more at five minutes. None of them had periventricular or intraventricular haemorrhage, periventricular leucomalacia, or any other abnormality on ultrasound scans carried out during the first two weeks of life, seizures, sepsis or infection of the central nervous system, or hyperbilirubinaemia requiring exchange transfusion. Infants with birth asphyxia, hydrocephalus, or congenital anomaly were excluded from the study. If needed, intensive care was provided in keeping with current concepts of thermal, metabolic, and ventilatory control. Nutrition in very low birthweight infants was provided by orogastric tube feeding of human milk or formula feed of 2·814 MJ/l.

ULTRASOUND SCANS

Infants were prospectively scanned with an Advanced Technology Laboratory (Bellevue) mechanical sector scanner and 7·5 MHz transducer. Scanning was carried out through the anterior fontanelle in the coronal and parasagittal planes, initially within 48 hours of birth, then daily during the first week of life and thereafter once a week for six weeks. Further scans were carried out serially at 4, 6, and 9 months, and—if the anterior fontanelle was still open—at 12 months. The coronal sonographic plane was used to measure the area of the lateral ventricles at the level of the mid-body in their occipital part just before they diverge. The plane of measurement was standardised by the alignment of the bodies of the lateral ventricles, the hippocampal gyri, and the pons that produce a vertical echogens band between the hippocampal gyri (fig 1). In a previous study we described the neuroanatomical landmarks that give the sonographic correlation to determine this plane. The measurements made at this level showed little interobserver error.[2]

Measurements were made using a digitising table (Numonics) connected to an Apple IIE personal computer. The instrument was calibrated at each of the five field settings. The images were enlarged by reference to the calibration, and the area enclosed by the lateral ventricles was calculated. Three measurements of the left and right lateral ventricle area were made at each study by a single examiner. The averages of the left and right area from each study were recorded for the analysis. The SEM of the successive measurements were less than 3%, suggesting that the reproducibility of the method was acceptable. The coronal and parasagittal images were examined for evidence of periventricular or intraventricular haemorrhage or periventricular leucomalacia. All images were evaluated independently by two specialists in paediatric ultrasonography.

MEASUREMENTS OF HEAD CIRCUMFERENCE

During each sonographic scan and clinic visit, the occipitofrontal circumference was measured with a paper measuring tape.

NEURODEVELOPMENTAL ASSESSMENT AND FOLLOW UP

Gestational age was determined from maternal

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dates and by the score developed by Dubowitz *et al.*[3] The babies were then examined by medical staff not concerned in the neonatal care at the ages of 4, 6, 9, 12, 18, and 24 months. The follow up examinations consisted of detailed neurological examination and an assessment of development using the Brunet-Lezine psychomotor development scale corrected for gestational age. This test is a French adaptation of the Gesell and Amatruda scale.[4 5] The items of the Brunet-Lezine test include assessment of posture, oculomotor coordination, language, and sociability. Evaluations of vision and hearing were also made. Neurodevelopmental outcome was classified as: normal; moderate neurological abnormalities (transient abnormality of tone); developmental delay (developmental quotient less than 80); cerebral palsy (spastic diplegia, quadriplegia, or hemiplegia); and neurosensory impairment (auditory or visual impairment). The results of the repeated neurological examinations between 18 and 24 months of age were the criteria for the presence or absence of neurodevelopmental abnormalities.

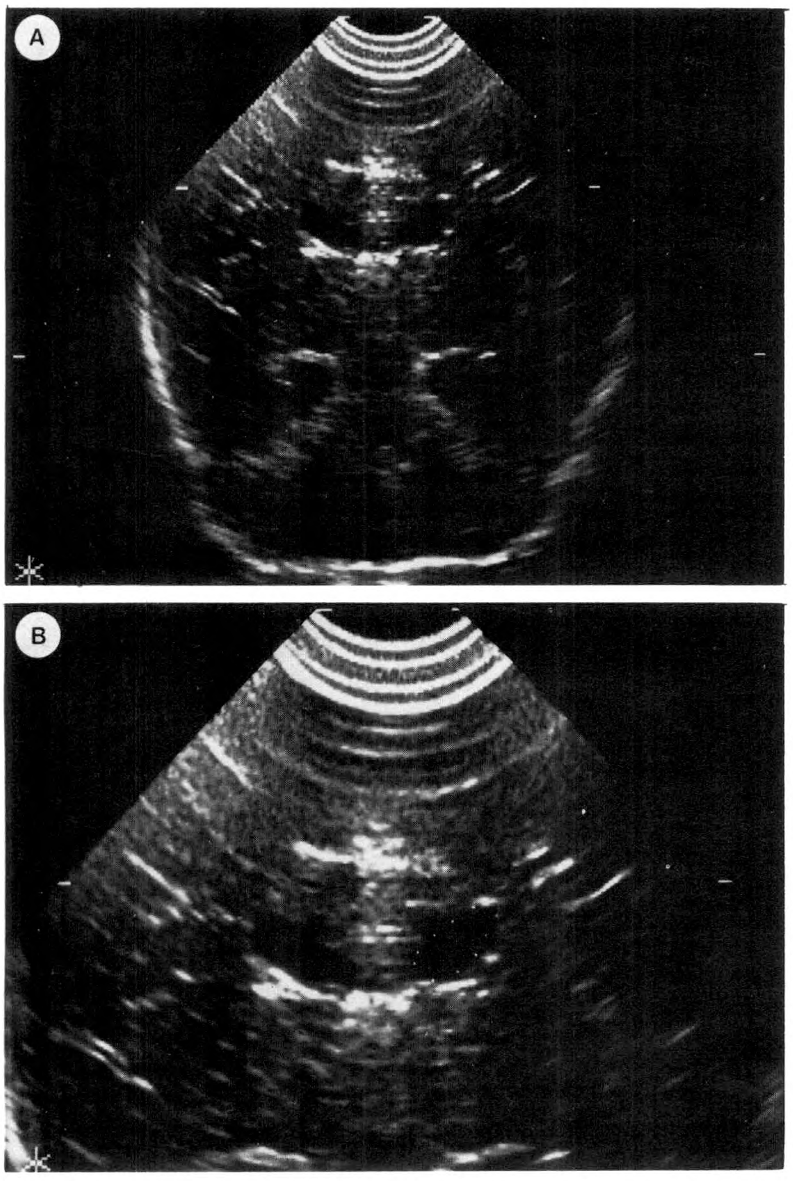

Figure 1 (A) Coronal ultrasound image at the level of the mid-body of the lateral ventricle; and (B) measurements obtained by digitising the ventricular area.

DATA ANALYSIS

Postnatal growth velocity (rate of growth) of occipitofrontal circumference and ventricular area, which is the slope of growth as a linear function of time of examination for each infant, was calculated. Regressions of occipitofrontal circumference on postnatal age, and of ventricular area on postnatal age, were calculated by considering the slope for each subject and then averaging the slopes. Linear regression analysis $(y=ax+b)$ by pooling all the data in the whole sample $(n=87)$ was used to evaluate the relation of ventricle area to gestational age, occipitofrontal circumference to postnatal age, and ventricle area to occipitofrontal circumference. Data are reported as mean (1SD), and a probability of $<0{\cdot}05$ was accepted as significant.

Results

Eighty seven preterm infants were included in the study. The birth weight, gestational age, and clinical details are shown in tables 1 and 2.

OCCIPITOFRONTAL CIRCUMFERENCE IN RELATION TO POSTNATAL AGE

The relation of occipitofrontal circumference to postnatal age was significant $(r=0{\cdot}80$; $p<0{\cdot}0001$; occipitofrontal circumference$=0{\cdot}44$ postnatal age$+28)$. The average of the slopes was 0·43 (0·12) cm/week. The mean postnatal growth velocity during the first six postnatal weeks was 0·53 (0·13) cm/week.

VENTRICULAR AREA IN RELATION TO POSTNATAL AGE

There was no variation in initial ventricular area as a function of gestational age at birth. $(r=-0{\cdot}027$; not significant). The mean birth ventricular areas ranged from 7·4 (3·6) mm^2 at 27 weeks' gestation to 8·5 (4·5) mm^2 at 36 weeks' gestation (not significant). For the rest of the study, therefore, ventricular area variation was calculated as a linear function of postnatal age. The mean (SD) at each postnatal age of examination is shown in fig 2. The average of the slopes was 0·34 (0·2) mm^2/week. The mean postnatal growth velocity during the first six postnatal weeks was 0·39 (0·19) mm^2/week. For

Table 1 Birth weights and gestational ages of 87 infants (51 girls and 36 boys)

Birth weight (g)	*No of infants*	*Gestational age (weeks)*	*No of infants*
800–1000	6	27–30	8
1001–1500	19	31–32	20
1501–2000	26	33–34	24
2001–2500	20	35–36	35
2501–3100	16		

Table 2 Details of 87 infants studied

Clinical details	*No of infants*
Hyaline membrane disease	26
Transient respiratory distress	26
Idiopathic apnoea	23
Patent ductus arteriosus	12
Assisted ventilation	34

the first six postnatal weeks the means (SD) for change/week in ventricular area, compared with the data from the first week, were 1·04 (2·05), 2·8 (2), 3·3 (2·7), 5 (3·7), and 5·84 (4) mm^2/week, respectively.

VENTRICULAR AREA IN RELATION TO OCCIPITOFRONTAL CIRCUMFERENCE

The relation of ventricular area to occipitofrontal circumference was significant ($r=0·55$; $p<0·0001$; ventricular area=0·75, occipitofrontal circumference=12). Figure 3 shows the regression line and 95% confidence intervals of the mean.

NEURODEVELOPMENTAL OUTCOME

The mean (SD) developmental quotient between 18 and 24 months was 101·7 (5). Five of the 87 children who were initially thought to be normal had developmental delay (developmental quotient=0·77 (0·01)). Five had transient abnormality of tone. None had cerebral palsy or neurosensory impairment.

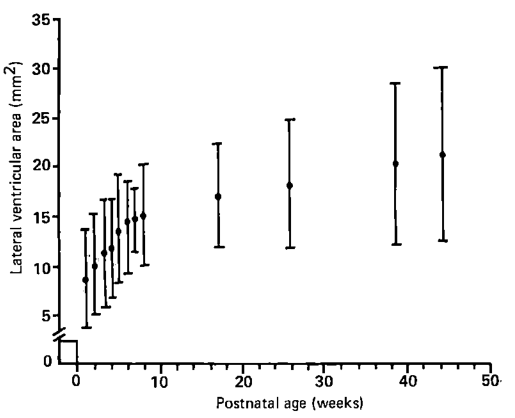

Figure 2 The mean (SD) of ventricular area at each postnatal age of examination.

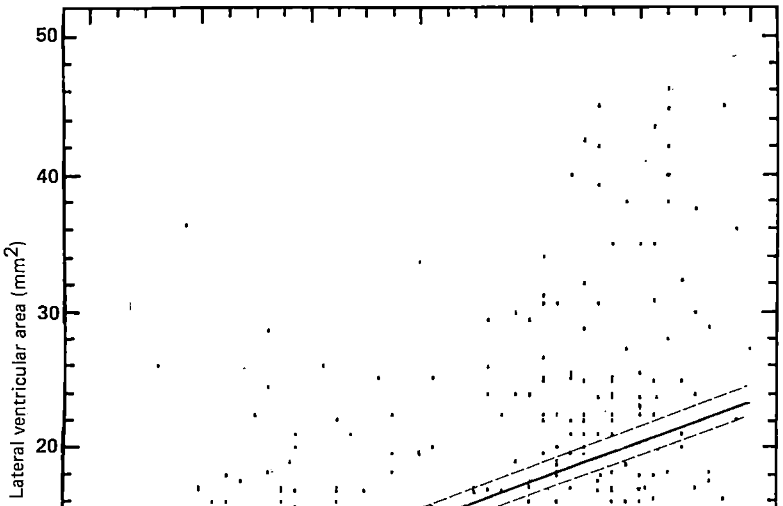

Figure 3 Mean lateral ventricular area and head circumference in 87 preterm infants showing the regression line (—) and 95% confidence intervals of the mean (===).

Discussion

The accuracy of cranial ultrasonographic measurements of brain ventricules has previously been established.[6 7] There is a close relationship between ventricle size measured by this technique and by computed tomography.[8] Most of the ultrasonographic studies of normal ventricle size in high risk neonates use transaxial width of the lateral ventricles and brain hemispheres.[9 10] The static images in these studies were obtained with a linear array transducer that transmits the ultrasound beam through the relatively thin temporoparietal region of the cranial vault in young infants. Using a 7·5 MHz sector scan that produces images with an angle of 90°, we found it difficult to obtain reproducible measurements with similar accuracy to those in the transaxial plane.

To calculate the ratio of transventricular diameter:intracranial hemidiameter, or transcranial diameter, a strong echo of the inner table of the skull is necessary. This is not always possible using real time sector scanning. The explanation lies in the fontanelle size which, in some cases, may not allow adequate transducer contact. Despite widespread use of various types of ratios, we believe direct measurement of the lateral ventricles is more accurate. Growth cannot be monitored reliably using a ratio, and abnormalities of both ventricle and cortex may not show as an abnormality when expressed as a ratio. Furthermore, when ventricular dilatation occurs, there is an increase in volume of cerebrospinal fluid, and linear measurements do not reflect this change. To date there are no data for the measurement of the cerebral ventricular system other than the lateral ventricles. The third ventricle is not usually seen on coronal scan if it is normal, and it must be dilated to be seen clearly. The fourth ventricle is difficult to measure because of its complicated shape and poor delineation on ultrasonography.[11] We chose to evaluate ventricular size on coronal scans quantitatively through the bodies of the lateral ventricles because of the reproducibility of these views. Such measurements are now possible using the echo analyser of most portable real time units.

We found no significant variation of initial ventricular area in relation to gestational age. One explanation is that during the first few days of life the lateral ventricles are small and slit like, and may be difficult to image. Their entire outline is often not seen on ultrasonography, rendering direct measurements unreliable. Sauerbrei *et al* reported that the lateral ventricles averaged 8·6 mm (range 9 to 11) in width between 25 and 35 weeks' gestation in normal preterm infants, with no variation as a function of gestational age.[12] By the end of the first week of life a rapid increase in ventricular size occurs, probably in association with the increase in the volume of cerebrospinal fluid. Changes from the fetal low pressure to the neonatal high pressure circulatory state that occur during this transitional period may possibly be accompanied by changes in the secretion or reabsorption, or both, of cerebrospinal fluid.

During infancy, increase in occipitofrontal circumference correlates well with increase in intracranial volume[13] and cellular brain growth.[14] The close relationship between the size of the lateral ventricles and the occipitofrontal circumference suggests that both variables change at a similar speed during extrauterine growth. By using a combination of measurements of occipitofrontal circumference and ventricular area it is possible to monitor changes in ventricle size in high risk preterm infants.

The most useful reference points for postnatal ventricle growth in preterm infants are established with knowledge of the subsequent developmental outcome of these infants. In our study, 94% of the infants had good outcomes at 2 years of age. The five infants with developmental delay (defined by a developmental quotient of less than 80) were not excluded from the study because neurologically they were no different from the rest of the sample. On the other hand, the relatively low developmental quotient (0·77) observed during this stage of the mental development of premature infants may be transitory and may not reflect definitive developmental retardation.[15]

These postnatal reference ranges developed for preterm infants who have good outcomes at 2 years should be used as the appropriate standards for growth of lateral ventricles.

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Area of lateral ventricles measured on cranial ultrasonography in preterm infants: association with outcome

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Abstract

The association between measurements of lateral ventricle area (determined by serial ultrasound scans) and outcome was studied in 70 preterm neonates of 33 weeks' gestation or less. The study group was subdivided into four groups according to cranial ultrasonographic findings at 2 weeks postnatal age: group A (n=15) had isolated periventricular/intraventricular haemorrhage; group B (n=20) had periventricular/intraventricular haemorrhage and dilated ventricles; group C (n=24) had periventricular/intraventricular haemorrhage and periventricular leucomalacia with or without dilated ventricles; and group D (n=11) had isolated periventricular leucomalacia. Eighty seven preterm infants with no evidence of intracranial disease and good neurodevelopmental outcomes at 2 years formed the control group. A poor outcome was observed in infants in group B, C, and D, particularly in those who had persistent dilated ventricles at 6 weeks postnatal age and extensive periventricular leucomalacia. There was no difference in outcome between group A and controls. During the first six weeks of life ventricular area growth velocities were significantly higher in groups B, C, D, compared with normal controls and group A. We suggest that persistent ventricular dilatation at this early stage carries a bad prognosis, which is the result of atrophy of the brain.

Periventricular/intraventricular haemorrhage and periventricular leucomalacia are relatively common complications in small preterm infants,[1 2] and the most common neonatal complication of these cerebral lesions is ventricular dilatation.[3] Palmer *et al*, and more recently Shankaran *et al*, have shown that infants with both periventricular/intraventricular haemorrhage and ventricular dilatation do not progress as well in their later development as infants with haemorrhage alone.[4 5] Little is known about the natural history of ventricular dilatation after periventricular/intraventricular haemorrhage in infants who do not develop clinical posthaemorrhagic hydrocephalus, and its association with neurodevelopmental outcome.

In a previous prospective study, we reported a reference range for ventricular area size and growth velocity in preterm infants.[6] The present longitudinal cranial ultrasonographic and clinical study was designed to assess (i) the growth velocity of the lateral ventricle areas during the first year of life in preterm infants who had periventricular/intraventricular haemorrhage with or without periventricular leucomalacia; (ii) the growth velocities of their head circumferences compared with the data obtained from normal controls; and (iii) whether successive measurements of ventricular area and early recognition of an increase in ventricular growth velocity are helpful in predicting neurodevelopmental outcome.

Patients and methods

Between October 1984 and July 1986, a total of 94 preterm infants of 33 weeks' gestation or less with ultrasonographic evidence of periventricular/intraventricular haemorrhage with or without periventricular leucomalacia during the neonatal period, were enrolled in a longitudinal cranial ultrasonographic and clinical study. Fifteen died in the neonatal period (haemorrhage grade IV: n=6, haemorrhage grade III: n=4, and leucomalacia: n=5). Four were lost to follow up (leucomalacia: n=1, haemorrhage grade II: n=1 and haemorrhage grade I: n=2). Five infants, with cerebral malformation (n=1), congenital anomalies (n=2), and meningitis (n=2) were excluded from the study.

The remaining 70 patients who survived for the duration of the study, were grouped according to ultrasonographic diagnosis at two weeks postnatally. Group A comprised infants with isolated periventricular/intraventricular haemorrhage alone; group B infants with periventricular/intraventricular haemorrhage and dilated ventricle but without periventricular leucomalacia; group C infants with periventricular/intraventricular haemorrhage and leucomalacia with or without dilated ventricles; group D infants with isolated periventricular leucomalacia.

Eighty seven normal preterm infants, with no intracranial abnormalities formed the control group. The reference range for their ventricular area size and growth velocity and their neurodevelopmental outcome have been previously reported.[6]

ULTRASOUND MEASUREMENTS

Infants were prospectively scanned with an Advanced Technology Laboratory (Bellevue) mechanical sector scanner and 7·5 MHz transducer. Scanning was carried out through the anterior fontanelle in the coronal and parasagittal planes, initially within 48 hours of birth, then serially at three to four day intervals until the age of 2 weeks, and thereafter once a week or more if necessary until discharge from hospital. Further scans were then performed serially at 2, 4, 6, and 9 months and, if the anterior fontanelle was still open, at 12 months.

The coronal sonographic plane was used to measure the area of the lateral ventricles at the mid-body level as previously described.[6] Ultrasonographic definitions and classifications, as well as diagnostic methods, remained uniform throughout the study. The severity of the haemorrhage was defined according to the four grade system of Papile *et al.*[7] Periventricular leucomalacia was defined according to the criteria established by Fawer *et al*,[8] and the sites of the changes were described separately and defined as frontal leucomalacia, occipital leucomalacia, frontoparietal leucomalacia, and frontoparieto-occipital leucomalacia in cases of extensive lesions affecting the entire periventricular white matter.

Early ventricular dilatation at two weeks postnatally was defined as the ventricular ratio of one or both lateral ventricles of greater than 0·37:1 (upper limit of normal for preterm infants) in at least two successive studies.[9]

Initial measurement of ventricular dilatation during the first two weeks of life was defined according to the ventricular ratio and not ventricular area, as the entire outline of the lateral ventricle is often not seen during the early stage of a periventricular/intraventricular haemorrhage particularly when a large amount of blood has filled the ventricle.

For follow up purposes the criteria we used to subdivide the babies into four groups were the maximum grade of haemorrhage or leucomalacia, and the presence or absence of ventricular dilatation at two weeks postnatally. Transient ventricular dilatation was defined as a temporary (less then six weeks) increase in size of lateral ventricles. Persistent dilatation was defined when still observed at six weeks.

NEURODEVELOPMENTAL ASSESSMENT AND FOLLOW UP

Gestational age was determined from maternal dates and the score developed by Dubowitz *et al.*[10] The babies were then examined by medical staff not concerned in the neonatal care, at the ages of 4, 6, 9, 12, 18, and 24 months. The follow up examination consisted of detailed neurological examination and an assessment of development using the Brunet-Lezine psychomotor development scale corrected for gestational age. This test is a French adaptation of the Gesell and Amatruda scale.[11] [12] The items of the Brunet-Lezine test include the assessment of posture, oculomotor coordination, language, and sociability. Evaluations of vision and hearing were also made. Neurodevelopmental outcome was classified as: normal; moderate neurological abnormalities (transient abnormality of tone); developmental delay (developmental quotient of less than 80); cerebral palsy (spastic diplegia, quadriplegia, or hemiplegia); and neurosensory impairment (auditory or visual impairment). The results of the repeated neurological examinations between 18 and 24 months postnatal age were the criteria for the presence or absence of neurodevelopmental abnormalities.

DATA ANALYSIS

Postnatal growth velocity (rate of growth) of occipitofrontal circumference and ventricular area, which is the slope of growth as a linear function of time of examination for each infant, was calculated. Regressions of occipitofrontal circumference on postnatal age, and of ventricular area on postnatal age, were calculated by considering the slope for each subject and then by averaging the slopes. Linear regression analysis ($y=ax+b$) by pooling all the data in the whole sample (n=70) and in each group (A, B, C, and D), was used to evaluate the relation of ventricular area to postnatal age, of occipitofrontal circumference to postnatal age, and of ventricle area to occipitofrontal circumference.

The score on the Brunet-Lezine scale corrected for gestational age and obtained at the latest examination between 24 and 30 months of postnatal age was used for analysis. One way analysis of variance was performed on the clinical characteristics and the Brunet-Lezine scale score to test the differences between each group. Analysis of variance for repeated measurement of ventricular area and head circumference growth velocities was performed to test the differences between each group.

Results

Seventy preterm infants were studied. Their clinical details and the distribution in centiles of their weight are shown in table 1. There were no significant differences among the four groups in

Table 1 Clinical details of the four groups studied. Values are expressed as mean (SD)

	Group			
	A (periventricular/ intraventricular haemorrhage) (n=15)	*B (periventricular/ intraventricular haemorrhage and dilated ventricles) (n=20)*	*C (periventricular/ intraventricular haemorrhage and periventricular leucomalacia) (n=24)*	*D (periventricular leucomalacia) (n=11)*
Birth weight (g)*	1445 (350)	1438 (288)	1342 (307)	1402 (327)
Gestational age (weeks)	31·9 (2)	31·5 (1·5)	30·3 (1·8)	31·2 (2)
Apgar score at:				
1 minute	7 (2)	6 (3)	6 (2)	7 (3)
5 minutes	8 (2)	7 (2)	6 (2)	8 (2)
No of days ventilated	11·87 (24·94)	15·71 (20·14)	21·8 (27·3)	14·4 (21·2)

*Centiles of the weight (g): 5° (900), 10° (1000), 15° (1050), 25° (1200), 50° (1400), 75° (1660), 85° (1740), 90° (1800), 95° (1850).

Table 2 Main ultrasonographic diagnoses

	Group			
	A (periventricular/ intraventricular haemorrhage) (n=15)	*B (periventricular/ intraventricular haemorrhage and dilated ventricles) (n=20)*	*C (periventricular/ intraventricular haemorrhage and periventricular leucomalacia) (n=24)*	*D (periventricular leucomalacia) (n=11)*
Grades of periventricular/intraventricular haemorrhage:				
I	8	7	10	NA
II	6	7	7	NA
III	1	6	6	NA
IV	0	0	1	NA
Periventricular leucomalacia:				
Frontal	NA	NA	14	3
Occipital	NA	NA	5	0
Frontal-parietal	NA	NA	4	1
Fronto-parieto-occipital	NA	NA	1	7
Dilated ventricles:				
Early (two weeks)	NA	20	14	0
Transient (<six weeks)	NA	12	6	0
Persistent (<six weeks or more)	NA	8	8	7

NA, not applicable.

birth weight, gestational age, Apgar score at 1 and 5 minutes, or duration of ventilator use.

The main ultrasonographic appearances in the four groups are shown in table 2. None of the 70 infants underwent repeat drainage of cerebrospinal fluid; in none was a shunt procedure carried out.

OCCIPITOFRONTAL CIRCUMFERENCE IN RELATION TO POSTNATAL AGE

The relation of occipitofrontal circumference to postnatal age was significant in the total group (n=70, r=0·79; p<0·0001; occipitofrontal circumference=0·47, postnatal age=26 weeks). There was no significant difference between the occipitofrontal circumference and postnatal age among groups A (r=0·76; occipitofrontal circumference=0·48, postnatal age=26 weeks), group B (r=0·82; occipitofrontal circumference=0·46, postnatal age=27 weeks) and normal controls (n=87, r=0·80; occipitofrontal circumference=0·44, postnatal age=28 weeks). Analysis of covariance showed that the regression lines of the occipitofrontal circumference/postnatal age association between controls and group C (r=0·45; occipitofrontal circumference=0·47, postnatal age=25 weeks) and group D (r=0·75; occipitofrontal circumference=0·42, postnatal age=25 weeks) were parallel; there was, however, a significant difference between controls and group C (p<0·0001) and between controls and group D (p<0·0001).

The average of the slopes of occipitofrontal circumference for the four groups was 0·44 (0·07) cm/week. During the first six postnatal weeks there was no significant difference in the mean (SD) occipitofrontal circumference growth velocity (cm/week) among the four groups and normal controls: controls=0·53 (0·13); group A=0·56 (0·13); group B=0·56 (0·21); group C=0·53 (0·10); and group D 0·50 (0·11).

VENTRICULAR AREA IN RELATION TO POSTNATAL AGE

The relation of ventricular area to postnatal age was highly significant among the four groups (n=70, r=0·45; p<0·0001; ventricular area=0·95, postnatal age=6·6 weeks). When group A and normal controls were compared (analysis of covariance), there was no significant difference in the ventricular area and postnatal age association. There was, however, a significant increase in ventricle size in relation to postnatal age in groups B, C, and D compared with both normal controls and group A (p<0·0001, analysis of covariance between groups B, C, D, and controls). Except for group A and controls, the regression lines of the ventricular area and postnatal age association were not parallel (fig 1).

The average of the slopes of ventricular area for the four groups during the first six postnatal weeks was 0·85 (1·02) mm^2/week.

The mean ventricular growth velocity (mm^2/week) during the first six postnatal weeks in each group is shown in table 3. There was no

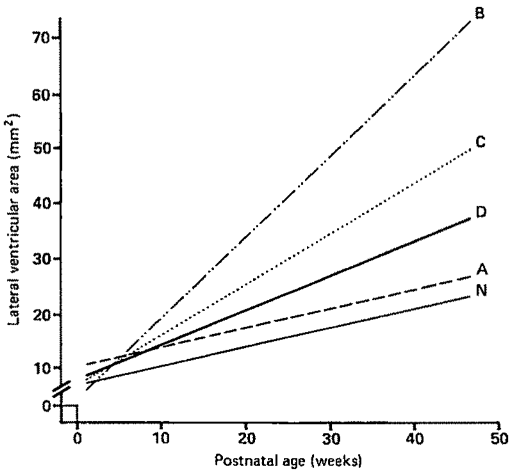

Figure 1 Regression lines of the area of the lateral ventricles during the postnatal period in the four groups compared with normal controls. N=normal controls (n=87, r=0·52, p<0·0001, ventricular area 0·38 mm^2, postnatal age=7 weeks); A=periventricular/intraventricular haemorrhage (n=15, r=0·32, p<0·0001, ventricular area=0·38 mm^2, postnatal age=10 weeks); B=periventricular/intraventricular haemorrhage and dilated ventricles (n=20, r=0·58, p<0·0001, ventricular area=1·53 mm^2, postnatal age=5 weeks); C=periventricular/intraventricular haemorrhage and periventricular leucomalacia (n=24, r=0·43, p<0·0001, ventricular area=0·96 mm^2, postnatal age=7 weeks); and D=periventricular leucomalacia (n=11, r=0·26, p<0·01, ventricular area=0·66 mm^2, postnatal age=8 weeks).

Table 3 Ventricular area growth velocity (mm^2/week) during the first six postnatal weeks. Values are expressed as mean (SD)

Group				
Controls (n=87)	*A (periventricular/ intraventricular haemorrhage) (n=15)*	*B (periventricular/ intraventricular haemorrhage and dilated ventricles) (n=20)*	*C (periventricular/ intraventricular haemorrhage and intraventricular leucomalacia) (n=24)*	*D (periventricular leucomalacia) (n=11)*
0·39 (0·19)	0·32 (0·3)	1·85 (1·07)	0·90 (0·45)	0·78 (0·64)

NS

p<0·0001

p<0·0004

p<0·0005

NS, not significant.

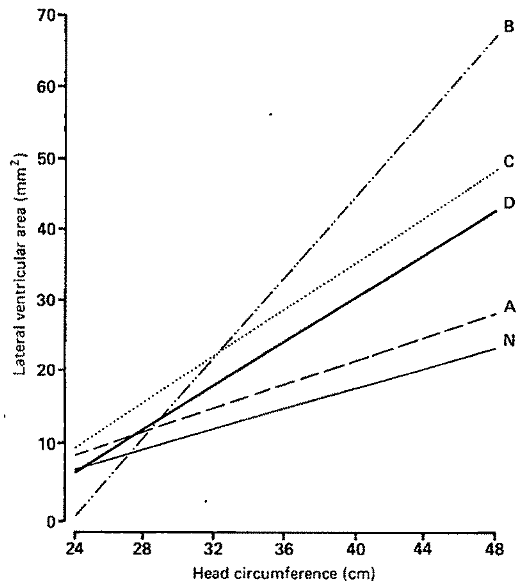

Figure 2 Regression lines of the area of the ventricles and head circumference in the four groups compared with normal controls. N=normal controls (n=87, r=0·54, p<0·0001, ventricular area=0·72 mm^2, occipitofrontal circumference=11 cm); A=periventricular/intraventricular haemorrhage (n=15, r=0·45, p<0·0001, ventricular area=0·85 mm^2, occipitofrontal circumference=12 cm); B=periventricular/intraventricular haemorrhage (n=20, r=0·61, p<0·0001, ventricular area=2·8 mm^2, occipitofrontal circumference=66 cm); C=periventricular/intraventricular haemorrhage and periventricular leucomalacia (n=24, r=0·45, p<0·0001, ventricular area=1·67 mm^2, occipitofrontal circumference=31 cm); and D=periventricular leucomalacia (n=11, r=0·36, p<0·0007, ventricular area=1·54 mm^2, occipitofrontal circumference=21 cm).

significant difference in the ventricular area growth velocity between group A and normal controls. The ventricular area growth velocity was significantly higher in groups B, C, D, compared with normal controls and group A.

VENTRICULAR AREA IN RELATION TO OCCIPITOFRONTAL CIRCUMFERENCE

Figure 2 shows the regression lines drawn for each group compared with that for normal controls. Infants in group A and normal controls had a similar ventricular area/occipitofrontal circumference association. Infants in group B had a significant (p<0·0001) increase in ventricular area/occipitofrontal circumference association compared with controls. Infants in groups C and D had a higher ventricular area/occipitofrontal circumference association compared with normal controls and group A (p<0·0001).

NEURODEVELOPMENTAL OUTCOME

Infants in group A did as well as normal controls. Infants in group B had a less good outcome: of the eight infants with persistent dilated ventricles at six weeks of life, seven had a development quotient <80, one had reduced visual activity. Poor outcomes were observed in infants in group C, particularly in the eight infants who also had persistent dilated ventricles; all those infants had development quotients <80. Of the two infants who developed cerebral palsy, one had extensive periventricular leucomalacia. Infants in group D had the worst outcome: of the seven infants with extensive lesions and persistent dilated ventricles, four developed cerebral palsy (table 4).

Perinatal factors such as gestational age, birth weight, Apgar score, number of days requiring ventilation, and neurological examination at discharge, did not relate significantly to outcome. Neurodevelopmental outcome related principally to two factors, namely persistent dilated ventricles at 6 weeks (p<0·001), and the presence of extensive periventricular leucomalacia (p<0·005), particularly if they were bilateral.

Figure 3 shows the distribution of ventricular

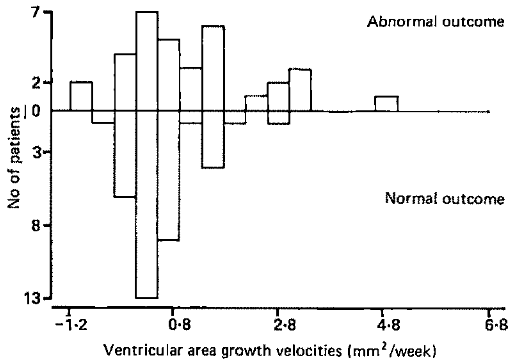

Figure 3 Ventricular growth velocities in relation to the neurodevelopmental outcome. Abnormal outcome (n=34)=developmental quotient <80, and normal outcome (n=36)=developmental quotient 80 or more.

Table 4 Neurological and developmental outcome of the four groups studied

	Group			
	A (periventricular/ intraventricular haemorrhage) (n=15)	*B (periventricular/ intraventricular haemorrhage and dilated ventricles) (n=20)*	*C (periventricular/ intraventricular haemorrhage and intraventricular leucomalacia) (n=24)*	*D (periventricular leucomalacia) (n=11)*
No with developmental delay (developmental quotient <80)	4	9	13	8
No with moderate neurological abnormalities	1	4	7	5
No with neurosensorial impairment	1	4	3	5
No with cerebral palsy	0	0	2	4

area growth velocities for the first six weeks in relation to the neurodevelopmental outcome.

Discussion

Infants in group A did not differ significantly from normal controls, though they kept slightly higher values of ventricular area throughout the study. In a cranial ultrasonographic study, Graziani *et al* showed that the width of the lateral ventricles and brain hemispheres in a group of preterm infants with periventricular/intraventricular haemorrhage without early ventricular dilatation did not differ significantly from those of preterm infants without evidence of haemorrhage.[13] D'Souza *et al* have shown that infants with haemorrhage alone had a similar ventricle width to biparietal diameter ratio compared with normal controls.[14] The favourable outcome observed in this group is in agreement with similar results reported by others.[5 7–13]

Dilated ventricles usually occur in infants who have sustained moderate to severe haemorrhage, and may be related to posthaemorrhagic obstruction. Both Palmer *et al* and Stewart *et al*, however, have occasionally noted ventricular dilatation after small haemorrhages in the subependymal area and within the ventricles.[4 5 7–15] Others have also described porencephalic atrophy resulting from haemorrhages in preterm infants.[16]

Dilated ventricles occurred even after small or moderate haemorrhage (group B). In infants with persistent dilatation, ventricular area remained significantly greater than in normal controls. There was no significant increase in cranial growth velocity despite the persistent increase of ventricular area. Although clinical signs and rapidly enlarging head circumference may be delayed or absent in small preterm babies with haemorrhage, the rate of growth of the occipitofrontal circumference in infants with posthaemorrhagic hydrocephalus in the absence of cerebral atrophy should be as great, if not greater, than that of infants without haemorrhage. Our results suggest therefore that posthaemorrhagic hydrocephalus alone is an insufficient explanation for dilated ventricles in some infants. Whether progressive brain atrophy is taking place or a decrease in early cranial growth related to the neonatal illness is occurring is not certain. Infants in group B with persistent dilated ventricles may have included those with brain atrophy as a result of diffuse hypoxic-ischaemic parenchymal damage not detected by ultrasonographic scanning, and subsequently associated with retarded development. Volpe *et al* have shown by positron emission tomography that periventricular infarction can be more extensive than the haemorrhage.[16]

In the 24 infants in group C and the 11 infants in group D, ventricular area growth velocity was significantly greater than that of normal controls; in addition, the rate of growth of occipitofrontal circumference remained significantly lower throughout the study, particularly in those with extensive lesions, suggesting diffuse cerebral atrophy with retarded development and cerebral palsy.

None of the infants with persistent dilated ventricles required shunts. In a recent study, Shankaran *et al* showed that the lack of ventricular decompression after ventriculoperitoneal shunt was strongly associated with poor outcome, suggesting that cerebral atrophy may have occurred before the insertion of the shunt in some infants with posthaemorrhagic ventricular dilatation.[5]

Early recognition of ventricular dilatation is important, as it seems to correlated with neurodevelopmental outcome. We chose to evaluate ventricular size quantitatively, because when ventricular dilatation occurs linear measurements do not reflect this change. Horbar *et al*, suggested that the lateral ventricular cross sectional area should be measured when dilatation occurred.[17] This was done in one infant with posthaemorrhagic hydrocephalus by planimetry of multiple coronal sections.

In our study, although the overlap among the groups is important, early determination of ventricular area growth velocities seems to be discriminatory for assessing outcome. The relatively increased value of the SDs of the slopes of the ventricular area growth velocity, is explained by the fact that some infants (group B) had rapid posthaemorrhagic ventricular dilatation.

We conclude that longitudinal cranial ultrasonographic follow up provides a useful assessment of brain in preterm infants. Comparison of direct ultrasound measurements (ventricular area) in the detection of changing ventricular size is helpful in planning treatment and in defining the relationship between neurodevelopmental outcome and cerebral lesions.

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Spontaneous resolution of bone mineral depletion in preterm infants

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Abstract

Fifteen preterm infants and 17 born at full term whose gestational ages ranged from 25 to 34 weeks and 38 to 42 weeks, respectively, were examined initially at postconceptional ages ranging from 38 to 44 weeks and subsequently at 46 to 71 weeks. Each examination included measurement of bone mineral content of the mid-forearm by single photon absorptiometry. For the preterm group, the mean (SD) value of bone mineral content at the first examination was 109·0 (27·6) mg/cm and was significantly lower than the corresponding value of 194·4 (19·6) mg/cm for the whole full term group. The mean subsequent rate of mineral accretion in the preterm group was 8·70 (4·60) mg/cm/week, the mean individual duration of observation being 9·7 weeks. Rate of mineral accretion for the full term group was independent of the duration of observation and averaged 1·60 (2·20) mg/cm/week. The difference between mean values of rate of mineral accretion in the preterm and full term groups was highly significant.

Our results show that there is a phase of rapid mineral accretion starting at 40 weeks' postconception in preterm infants that substantially reduces the perinatal mineralisation deficit.

We and others have previously shown that at 40 weeks' postconception the bones of preterm infants are substantially undermineralised compared with those of full term infants at birth.[1 2] The bone mineral deficit, assessed by single photon absorptiometry of the mid-forearm,[3] is roughly 20% in very low birthweight (<1500 g) infants,[1] and the deficit is even larger (about 35% after adjustment for crown-heel length or weight differences) in extremely low birthweight infants (<1000 g) born earlier.[2]

In a subsequent investigation in which we studied preterm and full term infants in the postconceptional age range 65 to 100 weeks and examined the results in the context of earlier observations we found that, by about 60 weeks, the bone mineral deficit that had been noted in preterm infants at 40 weeks had largely disappeared.[4] That conclusion, which we had not anticipated, was based solely on cross sectional observations. The data none the less strongly suggested that individual preterm infants must mineralise their bones rapidly in the period from 40 to 60 weeks' postconception, at a time when mineral accretion in full term infants is relatively slow.

In this paper we report our first longitudinal observations of mineral accretion in preterm and full term infants, which were made to test the hypothesis that, in many infants, osteopenia of prematurity is a transient condition that heals with time without direct clinical intervention.

Patients and methods

This study was approved by the local ethics committee. Thirty two infants born between 1984 and 1989 were examined. They were all white singletons whose parents gave informed consent for the investigations to be carried out, and who were prepared to bring their babies back to the neonatal unit for measurement when necessary. Of the 32, 15 infants (eight boys) had been born prematurely and comprised the 'preterm group—group P. The remaining 17 (nine boys), who were born after roughly 40 weeks' gestation, comprised the 'full-term group'—group F—and served as controls. Group F included a subgroup—group F1—that comprised seven infants (five boys). Those in group F1 were infants born at full term who were measured at postconceptional ages closely similar to those in group P (see below).

Infants in group P were born in or referred to our neonatal intensive care unit during a 36 month period that began in 1985. Twelve of the 15 were of appropriate weight for gestational age at birth, but three were small for dates. While in the unit, 12 were included in studies of the efficacy of various feeding regimens in promoting bone mineral accretion during the neonatal period (SW Ryan, J James, PJ Congdon, A Horsman, JG Truscott. Effect of vitamin D supplementation on bone mineralisation in preterm infants. Unpublished observations).[5] All infants had been fed with standard neonatal formulas from the onset of milk feeding until weaning, including four control infants from the mineral supplementation study who received 400 IU/day vitamin D supplementation throughout.[5] Eight infants had taken part in a vitamin D supplementation study (0·15 μg 1 α hydroxyvitamin D/day (n=3), 400 IU vitamin D/day (n=3), or 1000 IU vitamin D/day (n=2)), until the time of the measurement, which was about 40 weeks' postconception. After this those infants subsequently received 400 IU/day vitamin D. The remaining three infants were fed standard neonatal formulas plus 400 IU/day vitamin D. Infants received a maximum of 180 to 200 ml/kg/day of formula feed while in hospital, and although such intakes were recommended for infants at home they often received more. Absorptiometry was first performed

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Table 1 Descriptive statistics for gestational age, postconceptional age, weight, crown-heel length, and bone mineral content at the first and second observation times.

	Gestational age (weeks)	*At 39–44 weeks*				*At 46–71 weeks*			
		Postconceptional age (weeks)	*Weight (g)*	*Crown-heel length (cm)*	*Bone mineral content (mg/cm)*	*Postconceptional age (weeks)*	*Weight (g)*	*Crown-heel length (cm)*	*Bone mineral content (mg/cm)*
Group P:									
No of infants	15	15	15	12	15	15	13	11	15
Mean (SD)	29·07 (2·87)	40·89 (1·28)	2377 (599)	44·99 (3·76)	109·00 (27·60)	50·61 (3·05)	4028 (737)	54·11 (3·32)	191·67 (53·02)
Range	25·00–34·00	39·14–43·86	1550–3600	39·50–50·60	60·14–153·17	46·14–56·86	2670–5250	48·80–59·00	100·08–286·32
Group F:									
No of infants	17	17	17	17	17	17	17	17	17
Mean (SD)	40·24 (1·20)	40·73 (1·28)	3402 (405)	50·57 (1·71)	194·37 (19·62)	60·24 (7·41)	6895 (1253)	64·85 (4·56)	230·26 (56·16)
Range	38·00–42·00	38·43–42·71	2795–4270	47·30–54·50	165·25–221·21	47·14–70·29	4100–9400	53·10–71·00	168·75–370·79
Group F1:									
No of infants	7	7	7	7	7	7	7	7	7
Mean (SD)	40·57 (0·79)	41·02 (0·91)	3576 (382)	50·91 (2·33)	195·01 (21·81)	54·16 (3·35)	5938 (870)	61·29 (3·83)	206·81 (35·60)
Range	40·00–42·00	40·14–42·57	3050–4270	47·30–54·50	166·06–221·21	47·14–57·14	4100–6505	53·10–64·10	168·75–268·34

within days of discharge or during a return visit shortly afterwards. All returned for a subsequent measurement.

The 17 full term infants in group F were recruited from the local postnatal ward and were measured before discharge. All returned for a subsequent measurement. Those who were below the 10th centile for birth weight on standard charts were not entered in the study.[6] With five exceptions, all the infants born at full term received formula feeds from birth; the remainder were breast fed, with weaning starting at about 3 months.

Gestational age (completed weeks), calculated from maternal menstrual history and confirmed, for the preterm group, by either external examination,[7] or ophthalmoscopic examination of the lens,[8] had been documented in each case. For group P, gestational age ranged from 25 to 34 weeks (mean (SD) 29·1 (2·9) weeks), and for group F it ranged from 38 to 42 weeks (mean (SD) 40·2 (1·2) weeks). The mean gestational age of group F1 was 40·6 (0·8) weeks (table 1).

On two occasions bone mineral content (mg/cm) was measured at the middle of the forearm of each infant by photon absorptiometry as previously described.[1 3] The radiation exposure is small, and only a narrow area of the forearm is irradiated during a scan; skin entrance dose is 0·03 mGy/investigation. Weight (g) and crown-heel length (cm) were usually measured whenever a scan was performed.

In the postconceptional age range of 39 to 44 weeks, complete data (weight, crown-heel length, and bone mineral content were obtained in 12 of the 15 preterm infants in group P; in three infants crown-heel length was not measured. Results obtained at that time are referred to as 'term data'. All 15 infants in group P were later scanned in the period 46 to 57 weeks' postconception, when they attended for outpatient review. In two infants, neither weight nor crown-heel length was measured on the day of the second scan; in another two, crown-heel length was not measured. Results obtained at that later time are referred to as 'endpoint data'.

Soon after birth, in the postconceptional age range 38 to 43 weeks, complete term data weight, crown-heel length and bone mineral content were obtained for all full term infants in group F. All 17 infants were later scanned in the period 47 to 71 weeks' postconception, and the data obtained in that interval were also complete.

Infants in group F1 were selected from all full term infants (group F) and comprised those with both term and endpoint data acquired in the postconceptional age range 40 to 58 weeks; this range is closely similar to the range for group P (39 to 57 weeks). Descriptive statistics for the term and endpoint data are given in table 1 for groups P, F1, and F.

STATISTICAL ANALYSIS

Rate of mineral accretion was derived for each infant as the change in bone mineral content

Table 2 Rates of change of the measurements in the interval between the first and second observations

	Interval between first and second observations (weeks)	*Rate of weight gain (g/week)*	*Increase in crown-heel length (cm/week)*	*Rate of mineral accretion (derived from observed changes in bone mineral content (mg/cm/week))*
Group P:				
No of infants	15	13	11	15
Mean (SD)	9·72 (2·87)	178·7 (32·1)	0·970 (0·153)	8·7 (4·60)
Range	5·14–16·72	139·2–247·1	0·725–1·226	−1·04–17·64
Group F:				
No of infants	17	17	17	17
Mean (SD)	19·51 (7·08)	180·0 (38·2)	0·754 (0·097)	1·60 (2·20)
Range	7·00–28·00	102·9–232·1	0·562–0·900	−0·98–6·91
Group F1:				
No of infants	7	7	7	7
Mean (SD)	13·14 (3·19)	176·0 (46·7)	0·793 (0·065)	0·87 (1·97)
Range	7·00–17·00	102·9–232·1	0·686–0·868	−0·98–4·29

divided by the time interval between absorptiometric observations. Rates of weight gain and linear growth were similarly calculated. Group mean values of these and other variables were compared among groups using Student's *t* test.

Results

When mean values in group P were compared with those of groups F1 and F (table 1) at the start of the study (near 40 weeks' postconception) the preterm infants were significantly lighter than the full term infants, the difference between the means exceeding 1000 g (group P compared with group F1—difference 1200 g, $p<0{\cdot}001$; group P compared with group F—difference 1025 g, $p<0{\cdot}001$). The preterm infants were also significantly shorter, the difference between the means exceedng 5 cm (group P compared with group F1—difference 5·92 cm, $p<0{\cdot}002$; group P compared with group F—difference 5·58 cm, $p<0{\cdot}001$). The mean values of bone mineral content differed widely at the start of the study between the preterm and full term groups, the difference between the mean values of term data exceeding 80 mg/cm, or approximately 40% of the value in full term infants (group P compared with group F1—difference 86·0 mg/cm, $p<0{\cdot}001$; group P compared with group F—difference 85·4 mg/cm, $p<0{\cdot}001$).

Whereas mean values of postconceptional age at the start of the study were closely similar in all groups, they were dissimilar at the end (table 1). It was noted that despite the fact that those in group P were on average three weeks younger, 1900 g lighter, and 7 cm shorter, than those in group F1, the mean values of bone mineral content at the end were similar in the two groups (192 mg/cm and 207 mg/cm, respectively) and were not significantly different from one another.

The mean rates of weight gain (table 2) were closely similar in preterm and full term infants, averaging roughly 175 g/week; differences in the mean rates between groups P and F1, or P and F, were not significant. Longitudinal growth was more rapid in the preterm infants than the full term infants, however; the mean value of the rate of increase of crown-heel length was 0·97 cm/week in group P, which was significantly greater than the corresponding values of 0·79 cm/week for group F1 ($p<0{\cdot}02$), and 0·75 cm/week for group F ($p<0{\cdot}001$). In the preterm group the mean rate of mineral accretion was 10 times higher than in group F1, and five times higher than in group F; mean values of rate of

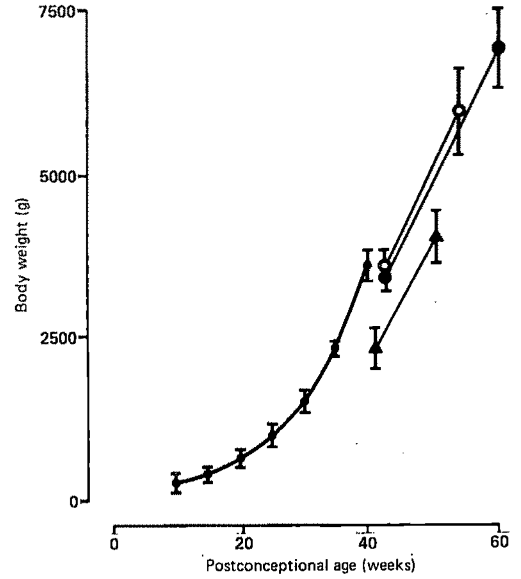

Figure 1 Body weight (g) in preterm infants (group P, solid triangles) and infants born at full term (group F1 and F, open and solid circles, respectively) in relation to postconceptional age. Points joined by straight lines represent the group mean (2 SEM) values measured at 39–44 weeks and 46–57 weeks, and are plotted at the group mean postconceptional ages. The curve approximates weight gain in utero.[10] Error bars on the curve indicate the expected mean (2 SEM) in relation to postconceptional age.

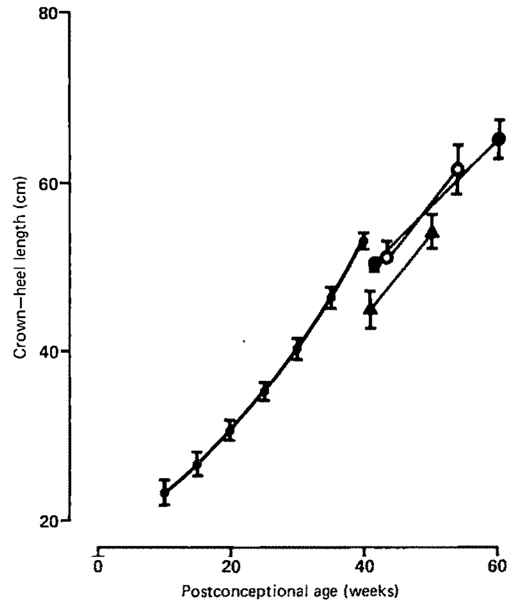

Figure 2 Crown-heel length (cm) in preterm infants (group P, solid triangles) and infants born at full term (groups F1 and F, open and solid circles, respectively). The curve approximates linear growth in utero.[10]

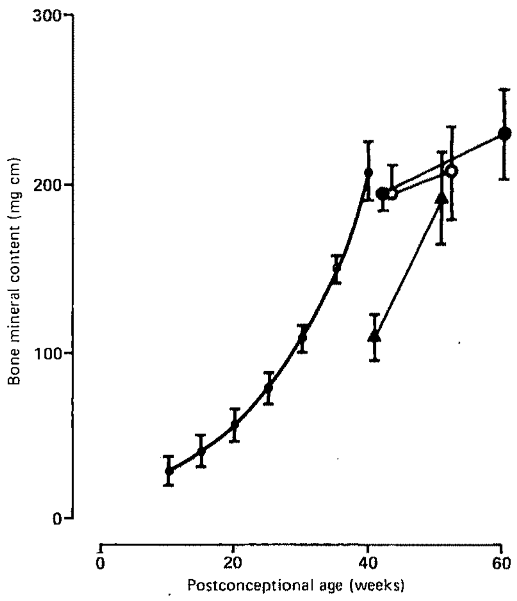

Figure 3 Bone mineral content (mg/cm) in preterm infants (group P, solid triangles) and infants born at full term (groups F1 and F, open and solid circles, respectively). The curve approximates bone mineralisation in utero.[10]

mineral accretion in groups P, F1, and F were 8·70 mg/cm/week, 0·87 mg/cm/week, and 1·60 mg/cm/week, respectively. Differences between mean rate of mineral accretion in groups P and F1, and groups P and F, were highly significant ($p<0·001$); the difference between mean rate of mineral accretion in groups F1 and F was not significant.

These results are displayed graphically in fig 1 (weight), 2 (crown-heel length), and 3 (bone mineral content). In each figure, the slope of the line joining the term and endpoint data for each group is closely similar but not identical to the mean of the individual rates of change given in table 2. Figures, 1, 2, and 3 also contain previously published curves based on observations of preterm infants near to the time of birth[9]; the curves approximate weight gain, linear growth, and bone mineralisation in utero.

Discussion

The results of our previous cross sectional study of preterm and full term infants in the postconceptional age range 65 to 100 weeks showed that if residual differences exist in bone mineral content between the groups, by 60 weeks those differences must be small in relation to the differences existing at 40 weeks.[4] On the basis of intermediate observations, we were able to narrow down a period within which rapid mineral deposition must occur in preterm infants, and we suggested that this period was between about 40 and 60 weeks.

The results of the present longitudinal study confirm that hypothesis. At a time between 40 and 60 weeks, when body weight and crown-heel length are increasing at about the same rate in preterm and full term infants, the rate of mineral accretion is of the order of five times greater in preterm than in full term infants. The changes we observed in preterm infants during that period are in stark contrast to the changes occurring before 40 weeks' postconception, and previously observed in other studies: from birth to 40 weeks, the net increase in bone mineral content is virtually nil in preterm infants.

In broad terms, our observations fit the following hypothesis. For an infant of any given weight and length there is an appropriate value of bone mineral content both in utero, and similarly after birth in a full term infant. When an infant is born prematurely, the in utero relationships are not maintained after birth, with the discrepancy between the infant's bone mineral content and that expected on the basis of weight and length increasing with time, at least up to 40 weeks' postconception. Thereafter, some mechanism comes into play that restores the relationship between bone mineral content, weight, and length in a preterm infant towards that present in full term infants of the same postconceptional age. Whatever the mechanism that tends to restore to normal the relationships between skeletal mineral content and body size, it is clearly inhibited until the time the preterm infants should have been born, and it would seem to have run its course by about 60 weeks' postconception.

With the measurements available it is impossible to disentangle two separate processes that might be occurring during the catch up phase. Those processes are the laying down of bone mineral on existing, previously undermineralised, bone matrix, and increasing overall cross sectional area of the bone at the measurement site, the bone tissue being rapidly and completely mineralised as the bone is laid down. If by 40 weeks' postconception, the bones of preterm infants are of normal cross sectional area (for length), then these and previously published data suggest that they must be undermineralised in the sense that the mineral density/unit volume of bone tissue is reduced. In the catch up phase, both processes could then be occurring in parallel. On the other hand, if by 40 weeks the bones of preterm infants are of reduced cross sectional area (for length), then it is possible that only the second process is operating in the catch up phase. In the first case, a disproportionately large amount of mineral must be deposited in relation to the new bone matrix being laid down. In the second case, bone matrix and mineral have to be rapidly deposited, but with the proportions expected in fully mineralised tissue. Given the familiar radiological appearance of 'rickets of prematurity', we believe it is likely, at least at the start (soon after 40 weeks), that the first process predominates, taking advantage of increased mineral supply to calcify undermineralised matrix. With normalisation of the mineralisation density of existing matrix, it is possible that—towards the later stages of the catch up phase—the second process might start to predominate.

Our findings are supported by the small amount of reported data about bone mineral content between 40 and 100 weeks' postconception in preterm and fullterm infants.[4] Helin *et al* conducted the only long term controlled follow up study in preterm infants, although the subjects were not recruited until 4 to 16 years old.[10] In that study children who had been born prematurely and with low birth weight, but at an unrecorded gestational age, were compared with historical controls. Among girls there was no difference in bone mineral content between preterm and full term children. Among boys bone mineral content was 7% lower ($p<0·01$) for age in the preterm group, but this difference disappeared when height or weight was taken into account. Dahlenburg *et al* found that preterm infants were not at increased risk of fracture in childhood,[11] although in infants of less than 33 weeks' gestation fractures occurred at a significantly earlier age. Gross found that the bone mineral content of the humerus of preterm babies weighing less than 1600 g at birth more than doubled between the postconceptional ages of 34 and 44 weeks. Between 34 and 37 weeks' postconception bone mineral content remained unchanged. The bone mineral content of preterm infants at 44 weeks' postconception exceeded that of infants born at full term and measured at 40 weeks postconception.[12]

The rapid mineral accretion seen between 40 and 50 weeks' postconception in preterm infants is accompanied by falling alkaline phosphatase activity and increasing phosphate concentration in plasma, which other authors have described in preterm infants over the same age range.[12–14] The mechanisms responsible for this rapid mineral accretion remain unknown. Studies in infants between 30 and 40 weeks' postconceptional age show a rising level of 1,25-dihydroxyvitamin D,[15] and increasing retention of dietary mineral with age.[16 17] Such mechanisms may be important, but further research during the appropriate postconceptional period is required. Hillman and Salmons postulated after investigating the relationship between 25-hydroxyvitamin D, calcium, and phosphorus levels in plasma, that classical vitamin D responsiveness only develops after 40 weeks' postconception in preterm infants.[18] Further research is required to validate this interesting hypothesis, which could certainly help to explain the rapid mineralisation.

We have often observed that infants who are discharged from our unit have increased their milk intake when they next attend the outpatient clinic. Intakes between 200 and 300 ml/kg/day are not uncommon compared with normal intakes of 150 to 180 ml/kg/day. The increase in intake is usually mediated by the hunger of the baby and obviously results in an increased dietary intake of mineral. In our study, although the rate of weight gain (g/day) was similar in full term and preterm infants, the weight gain relative to body weight (g/kg/day) is greater in the smaller preterm group. This could indicate a greater milk and hence mineral intake in these infants, which could also help to explain our observations, as increased mineral supply is known to increase bone mineral accretion.[5]

The mechanism that allows the rapid rate of mineral accretion, which we have seen, is of great interest. We intend to carry out further investigations to attempt to identify the underlying mechanism. It may then be possible to make use of the effect to benefit both preterm infants, with some of the more serious manifestations of osteopenia of prematurity, and other groups of patients with failure of mineralisation.

Dr Peter J Congdon died tragically at the early age of 44 while this work was being carried out. His sustained dedication to the group's work on osteopenia of prematurity will always be remembered with admiration by the other authors. All felt that, in view of the importance of the findings reported above, it would be an appropriate tribute to dedicate this paper to him, which we do with a deep sense of affection and respect.

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Preoperative stabilisation in congenital diaphragmatic hernia

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Abstract

Between January 1983 and November 1986, 26 newborn infants with congenital diaphragmatic hernia were treated by early operation at a mean of 7 hours of age. A further 23 infants admitted between December 1986 and December 1989 were stabilised for a mean period of 40 hours before operation. There was no significant difference in survival between the two groups. Delayed operation is not detrimental to infants with congenital diaphragmatic hernia.

It is traditional for congenital diaphragmatic hernia to be repaired as an emergency procedure. The rationale of this approach is that removing the abdominal viscera from the chest allows expansion of the lungs with improved ventilation.[1] It has never been proved, however, that emergency repair of congenital diaphragmatic hernia is beneficial.

A disadvantage of early operation is that the surgical stress superimposed on an already unstable patient with congenital diaphragmatic hernia can precipitate or aggravate pulmonary hypertension. It was therefore suggested that delayed operation preceded by a period of stabilisation might be beneficial because of the decreased risk of pulmonary hypertension.[1–3] Cartlidge *et al* reported improved survival after stabilisation for four to 16 hours before surgery,[2] but this has not been confirmed by others.[1 3] The purpose of this study was to assess the outcome in a group of patients who had delayed operation with preoperative stabilisation compared with that in patients who had conventional emergency repair of the congenital diaphragmatic hernia.

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Patients and methods

All patients with congenital diaphragmatic hernia admitted to Royal Liverpool Children's Hospital, Alder Hey between January 1983 and December 1989 were studied. The case notes were reviewed and gestational age, age at the onset of symptoms, age at operation, duration of preoperative stabilisation (if any), survival, and necropsy data on those who died were extracted.

Group 1 comprised the patients admitted between January 1983 and November 1986 all of whom underwent conventional emergency repair of congenital diaphragmatic hernia. The patients in group 2 were admitted between December 1986 and December 1989; these were stabilised before operation by paralysis with pancuronium, mechanical ventilation, intravenous infusion of crystalloids and colloids, and monitoring of blood gas tensions and ventilation. Pharmacological support, which included tolazoline, dopamine, and dobutamine, was used as indicated. Infants who did not improve clinically during the period of stabilisation were not operated on. After operation the supportive measures mentioned above were continued as indicated for patients in both groups. The differences between the two groups were compared with the Student's *t* test and χ^2 test; probabilities of <0·05 were considered significant.

Comparison of two groups of newborn infants with congenital diaphragmatic hernia. Values are expressed as number (%) unless otherwise stated

	Group 1 (early operation) (n=26)	*Group 2 (delayed operation) (n=23)*
Presentation:		
<6 hours	25 (96)	21 (91)
6–12 hours	1 (4)	2 (9)
Gestational age <38 weeks	3 (12)	3 (13)
Mean (range) birth weight (g)	2760 (947–4000)	2877 (1000–4100)
Associated major anomaly	2 (8)	3 (13)
Right sided hernia	3 (12)	4 (17)
Age at operation (hours):		
Mean (SD)	7 (3)	47 (40)*
Range	4–18	12–168
Total No who survived	14 (54)	11 (48)

*p<0·001.

Results

There were 49 patients: 26 in group 1 and 23 in group 2. The patients in the two groups were similar in terms of birth weight, maturity, side of defect, age at presentation, and incidence of associated anomalies (table).

Five patients died before operation in group 1 (19%) and seven in group 2 (30%); this was not significant. The remaining 37 patients underwent surgical repair of the diaphragmatic hernia and the results are summarised in the table. The mean age at operation in group 1 was 7 hours, and in group 2 was 47 hours. The survival rates of 54% and 48%, respectively, were not significantly different.

Necropsies were carried out on 15 infants, in nine of whom total lung weights were available. The mean (SD) total lung weight was 28 (17·07) g in group 1 (n=4) and 17 (8·56) g in group 2 (n=5); this was not significant. Normal total lung weight for a newborn is 60 (15) g.[4]

Discussion

It has long been appreciated that there can be deterioration even after the congenital diaphragmatic hernia has been repaired. Meticulous cardiopulmonary support of the neonate is critical and may improve survival in some cases.[5 6] Pulmonary hypoplasia and persistent pulmonary hypertension are two important factors that determine the outcome in neonates with diaphragmatic hernias.[1 2] Additional factors include associated congenital and chromosomal abnormalities.[7] There is no treatment for pulmonary hypoplasia at present, apart from early prenatal repair of fetal diaphragmatic hernia which remains highly experimental, and neonatal lung transplant (which may be an option for the future). Any improvement in survival rates, therefore, should take account of factors that are likely to moderate pulmonary hypertension. Langham *et al* reported from the extracorporeal membrane oxygenation (ECMO) central registry that survival in critically ill neonates with a predicted 100% mortality was 58% when ECMO was used.[8] ECMO improves survival mainly by resting the lungs and, possibly, reducing the risk of pulmonary hypertension. ECMO is not yet available in the United Kingdom. The appreciably lower total lung weight in most of our patients who died, particularly in group 2, indicates that pulmonary hypoplasia was the main cause of death.

Cartlidge *et al* reported that survival improved from 12·5% after early operation (done as soon as possible after birth) to 52·9% after stabilisation for four to 16 hours before operation.[2] This relatively short stabilisation period may not be adequate in haemodynamically labile patients and it is difficult to explain the impact of such a policy on survival rates. Furthermore, a survival rate of 12·5% after early operation is much lower than others have reported.[3 6] Langer *et al* reported 61 neonates from Toronto and found a mortality of 58% in the early surgery group (n=31) and 50% in the delayed surgery group (n=30).[3] From these results, which were not significantly different, they concluded that diaphragmatic hernia repair should be done only when the patient is satisfactorily stabilised. Our findings of 54% and 48% survival in the early and delayed surgery groups, respectively, corroborate the observations of Langer *et al.*

There are a number of possible reasons for the similar survival rates in our two groups of patients. Firstly, the mean lung weights of infants who died did not differ significantly between the two groups, probably because of the small numbers involved; they were, however, lower in group 2, implying that these were higher risk patients than group 1. Secondly, prenatal ultrasonography has led to earlier diagnosis and transfer to neonatal surgical centres of infants with a poor chance of survival.[5 9] This may have been a factor affecting improved survival in group 2, but we do not have adequate data to confirm this.

In summary, in patients with congenital diaphragmatic hernia, overall survival rates after preoperative stabilisation and delayed surgery are at least comparable with the results of early operation. It is possible that continued evaluation of preoperative stabilisation and delayed surgery may bring about an improvement in survival and also avoid operations on those who are not likely to survive.

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Peak inspiratory pressure requirements in infants born weighing less than 750 g

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Abstract

The possibility that peak inspiratory pressure requirements or the arterial:alveolar oxygen ratio can predict the clinical outcome in infants weighing <750 g at birth was explored in a consecutive series. Nine of 10 infants (90%) with a peak inspiratory pressure requirement of more than 18 cm H_2O at 48 hours or more than 16 cm H_2O at 72 hours from age subsequently died later of respiratory causes (defined as death after 72 hours of pulmonary interstitial emphysema, bronchopulmonary dysplasia, or cor pulmonale). Twenty of 21 remaining infants (95%) survived until discharge. Using these data a 95th centile for peak inspiratory pressure requirement during the first 72 hours of life was constructed. The potential value of this centile in predicting later death of respiratory causes was examined in a separate series. Twelve of 15 infants (80%) whose peak inspiratory pressure requirements remained below the 95th centile, or were not ventilated (n=6), survived. In contrast, 11 of 12 (92%) infants whose requirements crossed the 95th centile died later of respiratory causes. The infants who died had more radiological changes and higher mean arterial carbon dioxide pressure than survivors suggesting that the severity of the initial lung disease rather than the way that ventilation was managed determined prognosis. Peak inspiratory pressure requirement was more useful than arterial:alveolar oxygen ratio in clearly distinguishing between survivors and infants who died later of respiratory causes.

Neonatal intensive care is progressively being applied to smaller, less mature infants.[1] Many who weighed <750 g at birth now survive; their neurodevelopmental outcome and social and financial costs have previously been reported.[2–10] All centres report high mortality, however, particularly during the first 72 hours of life. Infants who survive this initial period and subsequently die, although only a small percentage of live births, cause great concern because they often undergo prolonged intensive care, have multiple complications, and cause distress to families and staff.[11 12] The severity of early lung disease is an important determinant of survival in extremely low birthweight (ELBW) infants.[13] As most infants who weigh <750 g at birth are ventilated, we examined the possibility that ventilation requirements in the first 72 hours of life predict those infants at high risk of dying later of respiratory causes (death after 72 hours age from pulmonary interstitial emphysema, bronchopulmonary dysplasia, or cor pulmonale).

Few previous attempts have been made to identify predictors of late respiratory death. Zarfin *et al* retrospectively studied a selected group of infants who weighed less than 801 g at birth up to the age of 28 days, and was able to predict survival with an accuracy of only 79%.[14] In a population of infants whose median birth weight was 970 g, Kimble *et al* found that infants who died consistently required higher ventilator pressures, rates, and oxygen concentrations than infants who survived, although the two groups could not clearly be distinguished by ventilator data alone.[15] From clinical experience in our nursery we felt that a clear distinction might arise if only those infants who weighed less than 750 g at birth were considered. Our objective was to determine, in a consecutive series, if this clinical impression could be confirmed. The ventilation requirements of a group of infants were therefore studied to see if we could identify correlates with survival. These correlates were then applied to a separate group to determine their prognostic value. A retrospective design ensured that the study had no influence on clinical management.

Patients and methods

During the period of the study, British Columbia's Children's Hospital special care nursery was the only tertiary neonatal intensive care unit for an average of more than 42 000 deliveries/year in the province. All the infants born in hospital were delivered at the Grace Hospital delivery suite adjacent to the special care nursery. Other infants were referred from hospitals throughout British Columbia.

All infants born alive weighing <750 g, whose gestational age was 20 weeks or more, and who survived for at least 72 hours, were eligible for the study. We decided to include only those infants whose principal cause of death was respiratory and for whom reliable documentation was available. Exclusion criteria therefore were: death after 72 hours from non-respiratory complications, and admission to the special care nursery after 6 hours of age. We performed two studies; the purpose of the first was to generate criteria that clearly distinguished survivors from infants who died later of respiratory causes, and that of the second was to test the criteria in a separate group of consecutive births.

FIRST STUDY

In the first study we examined data from 115

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infants born between 1 January 1985 and 31 December 1986, 111 of whom (97%) were born in the hospital and four of whom (3%) were referred. Fifty three of those born in hospital (48%) died in the delivery suite. Twenty eight of the remaining 62 infants who were admitted to the special care nursery died before they were 72 hours old. The 34 infants (30% of the initial 115) were still alive at 72 hours fulfilled the entry criteria. Three who died after 72 hours of non-respiratory causes (two of sepsis and one of a large intracerebral haemorrhage) were excluded from analysis. The remaining 31 infants, 21 survivors and 10 who subsequently died from respiratory complications, were studied in detail.

SECOND STUDY

In the second study we examined 60 infants born between 1 January 1987 and 31 December 1987, 58 of whom (97%) were born in the hospital and two of whom (3%) were referred. Twenty two (38%) infants died in the delivery suite. Nine of the remaining 38 infants who were admitted to the special care nursery died before they were 72 hours old. Twenty infants (48% of the initial 60) who were still alive at 72 hours fulfilled the entry criteria. Two who died after 72 hours of non-respiratory causes (one from sepsis and one from a large intracerebral haemorrhage) were excluded from analysis. The remaining 27 infants, 13 survivors and 14 who subsequently died from respiratory complications, were studied in detail.

VENTILATOR MANAGEMENT

Babies were initially ventilated with a positive end expiratory pressure of 3–5 cm H_2O and at the lowest peak inspiratory pressure that produced clinically adequate chest expansion. The inspiratory time was usually set at 0·5 seconds but the initial ventilator rate varied. Settings were subsequently adjusted to produce an arterial carbon dioxide pressure ($PaCO_2$) in the range of 4·7–6·0 kPa and the arterial oxygen pressure (PaO_2) in the range of 8·0–10·7 kPa. The Sechrist model IV-100, the Bear Cub BP2001, or the Bourns BP200 ventilator was used. No infant in either study received surfactant.

SOURCES OF DATA

Clinical details of the obstetric and neonatal course were transferred from the medical records on to standardised forms. Gestational age was determined from the date of the mother's last menstrual period or, if this was not known, by the result of the early obstetric ultrasound scan. The neonatologist's assessment was used where this differed from other estimates of gestational age by more than two weeks (n=1) and where other estimates were not available (n=6). In our nursery ventilator measurements are routinely recorded on to flow charts at the time of blood gas sampling. From these charts we abstracted simultaneous values of fractional inspired oxygen (F_IO_2), peak inspiratory pressure, blood gas values, and site of blood sampling, obtained nearest to the following ages (hours) and within the range stated in parentheses 6 (2), 12 (2), 18 (3), 24 (3), 36 (4), 48 (4), and 72 (8). The follow up clinic records were examined in order to find out the current clinical condition of infants in the first population.

For the first study changes on the initial chest radiograph (taken at 1·8 (0·7) hours) were graded by a consultant paediatric radiologist who was unaware of the clinical outcome according to the following classification: grade 1=clear; 2=mild granularity, with or without air bronchograms; 3=granularity or haziness that obscured the heart border, with or without air bronchograms; and 4=diffuse opacity. The presence of pneumothorax or pulmonary interstitial emphysema, or both, on any film taken during the first 72 hours of life was noted.

ANALYSIS OF DATA

Peak inspiratory pressure was used as an index of ventilation requirement and arterial:alveolar oxygen ratio as an index of gas exchange.[16] In the first group, survivors were compared with infants who died later of respiratory causes to find out whether the two groups could be separated on the basis of their peak inspiratory pressure requirement at any of the age intervals reviewed. We chose the 95th centile peak inspiratory pressure requirement (defined as the value of peak inspiratory pressure that was more than that required by 95% of the survivors) as a cut off value. Only after this cut off was established were peak inspiratory pressure requirements examined in the infants who died later of respiratory causes.

In the first study, arterial:alveolar oxygen ratios were calculated when arterial blood gas values were available (193 of the 217 blood gas results reviewed). The following equation was used:

$$\text{arterial:alveolar oxygen ratio} = PaO_2/(713 \times F_IO_2) - (RQ \times PaCO_2)$$

The respiratory quotient (RQ) was taken as 1·0. This was justified because energy is derived from the using up of glycogen stores and from carbohydrate given during the first 72 hours of life. As with peak inspiratory pressure requirements, the 95th centile arterial:alveolar oxygen ratio (defined as the value of arterial:alveolar oxygen ratio that was less than that of 95% of survivors) was chosen as a cut off point. Values in the infants who died later of respiratory causes were then examined.

Results

FIRST STUDY

The birth weights, gestational ages, and sex distribution of all the infants who were potentially eligible for the study at the time of birth or transfer to the special care nursery are shown in table 1. As expected, infants who died in the delivery suite were extremely small and immature. Infants who died early (less than 72 hours old) or later (over 72 hours old) tended to have lower birth weights than those who survived to be discharged. During the study period no

Table 1 Birth weights, gestational ages, and sex distribution in the two groups

	Total No	*No of boys*	*Mean (range) birth weight (g)*	*Mean (range) gestational age (weeks)*
First study:				
Live births in hospital	111	52	542 (260–740)	23·6 (20–33)
Live births from elsewhere	4	2	548 (450–600)	23·3 (23–24)
Died in labour and delivery room	53	26	450 (260–730)	22·1 (20–27)
Total admissions	62	28	621 (420–740)	24·8 (22–33)
Early deaths	28	16	609 (440–720)	24·5 (22–33)
Survivors	21	8	660 (570–740)	25·0 (23–28)
Late deaths from respiratory causes	10	3	590 (420–720)	25·6 (24–28)
Second study:				
Live births in hospital	58	31	576 (200–745)	24·2 (20–33)
Live births from elsewhere	2	0	644 (560–727)	24·0 (24–24)
Died in labour and delivery room	22	9	447 (200–700)	22·0 (20–29)
Total admissions	38	22	654 (360–745)	25·4 (23–33)
Early deaths	9	5	601 (360–690)	24·2 (23–28)
Survivors	13	6	684 (480–745)	26·2 (24–33)
Late deaths from respiratory causes	14	10	666 (560–730)	25·5 (24–28)

infant of gestational age of less than 23 weeks or having a birth weight of less than 570 g survived. The sex distribution among those who survived and those who died later of respiratory causes was similar. Of the 31 study infants, 21 survived until discharge and 10 died from respiratory complications. The median length of survival among those who died was 31·5 days (range 4–289 days); four survived for four months or longer. The 21 babies who survived (18% of the initial population of 115) were discharged at a median age of 140 days, range 54–417 days. All had been seen at the Children's Hospital follow up clinic at a mean corrected age of 15 months (range 8–21). Twenty infants were still alive; one had died of a cause not related to prematurity (metastatic rhabdomyosarcoma of the mandible).

Thirty three of the 34 infants who survived until they were 72 hours of age were ventilated throughout that period. One infant was electively extubated at 12 hours of age and discharged alive. There was more scatter in peak inspiratory pressure values among the infants who died later of respiratory complications than among the survivors, suggesting that their ventilation requirements were more unstable. The median peak inspiratory pressure values were lower among survivors than those who died later, and the two groups became more distinct with increasing age (fig 1). The 95th centile peak inspiratory pressure requirement in this group was 24 cm H_2O at 6 hours age and decreased to 16 cm H_2O by 72 hours age (fig 1 (B)). At the 48 and 72 hour time points only one of 10 infants who died later had a peak inspiratory pressure requirement below the 95th centile and (by definition) only one of the 21

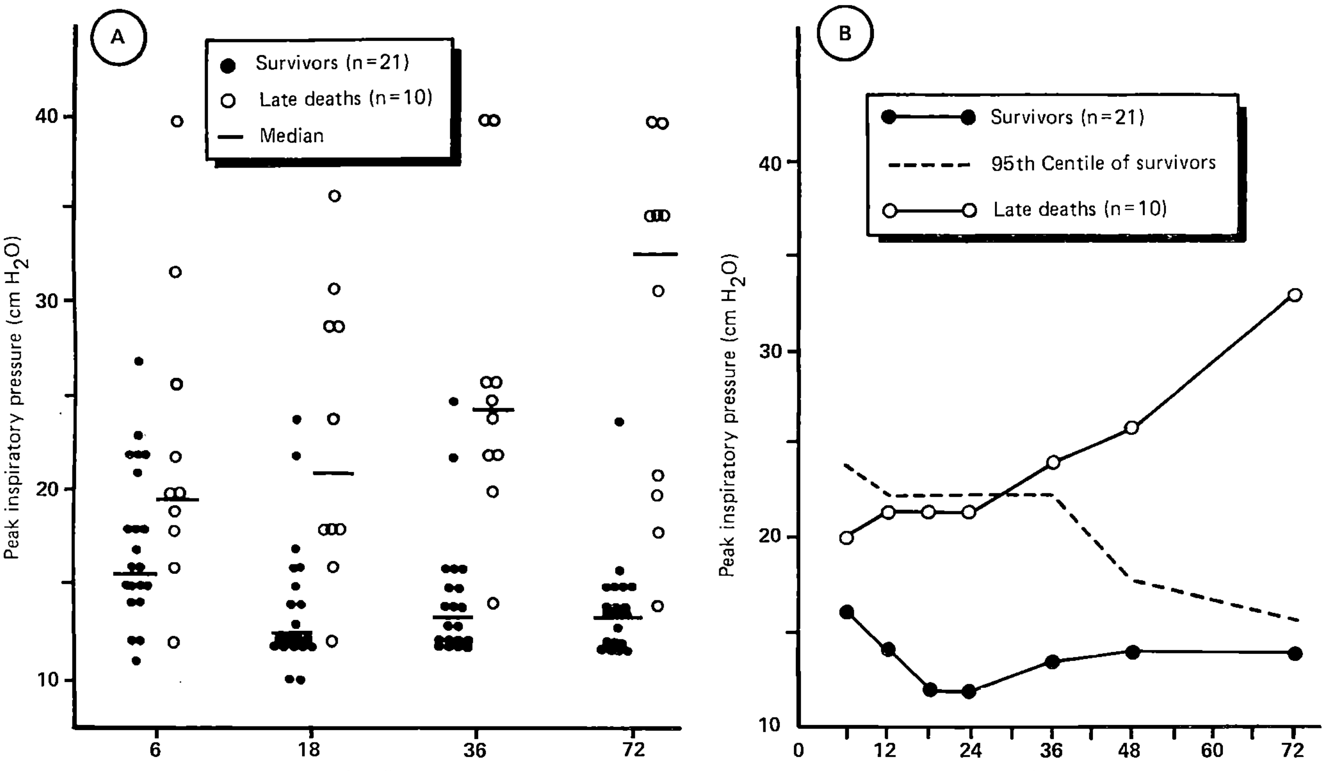

Figure 1 (A) The range and distribution of peak inspiratory pressure requirements during the first 72 hours of life in survivors and infants who died later of respiratory causes in the first study. For clarity, values at 12, 24, and 48 hours have been omitted. Figure 1 (B) The median peak inspiratory pressure requirements in the survivors and those that died later, and the 95th centile peak inspiratory pressure for the survivors. One survivor was extubated before 18 hours.

survivors had a peak inspiratory pressure requirement above the 95th centile. The 95th centile therefore seemed to be a good discriminant for survival among infants in the first study group.

There was a wide distribution in the values of arterial:alveolar oxygen ratio: 0·1–0·8 among survivors and 0·05–0·4 among those who died later. The two groups became more distinct with increasing age, but not to the same degree as was seen with the peak inspiratory pressure values. The 95th centile arterial:alveolar oxygen ratio varied between 0·1 and 0·2 during the first 24 hours, and then remained stable around 0·19 until 72 hours of life. By 48 hours of life, three of nine infants (33%) among those who died later and for whom data was available had an arterial:alveolar oxygen ratio above the 95th centile. One survivor (by definition) had an arterial:alveolar oxygen ratio below the 95th centile. Thus the 95th centile arterial:alveolar oxygen ratio was not as good a discriminant for survival as the 95th centile peak inspiratory pressure requirement. For this reason we chose the 95th centile peak inspiratory pressure requirement for assessment in the second study.

SECOND STUDY

We applied the survival criteria of a peak inspiratory pressure requirement below the 95th centile by 72 hours of life, established in the first study, to 27 infants born between 1 January 1987 and 31 December 1987. Their birth weights, gestational ages, and sex distribution (table 1) were similar to those in the first study. The peak inspiratory pressure requirements at 72 hours of the 13 infants who survived (median at discharge 124 days) and those of the 14 infants who died later of respiratory causes (median age of death 138 days) are shown in fig 2. Of 13 infants whose peak inspiratory pressure requirement crossed and remained above the 95th centile during the first 72 hours of life, 12 died of respiratory complications. Thus the predictive value for late respiratory death of a peak inspiratory pressure requirement above the 95th centile was 92% ($p=0·00017$, two tailed Fisher's exact test). In contrast, 12 (80%) of 15 infants whose peak inspiratory pressure requirement remained below the 95th centile, survived and three (20%) died.

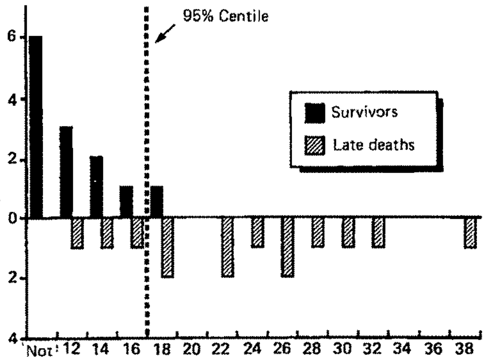

Figure 2 Peak inspiratory pressure requirements at 72 hours for the 13 survivors (solid bars) and the 14 infants that died later of respiratory causes in the second study. The 95th centile peak inspiratory pressure requirement for infants in the first study is also shown.

Table 2 Radiological changes

	No (%) of early deaths	*No (%) of survivors*	*No (%) of late deaths from respiratory causes*
Radiographic changes:			
Grade 1	3 (11)	12 (57)	2 (15)
Grade 2	6 (21)	8 (38)	1 (8)
Grade 3	15 (54)	0	7 (54)
Grade 4	1 (4)	0	2 (15)
Pulmonary interstitial emphysema	16 (57)	3 (14)	10 (77)
Pneumothorax	8 (29)	2 (10)	3 (23)
No radiograph taken	3 (11)	1 (5)	1 (8)
Total	28 (100)	21 (100)	13 (100)

In the first study pulmonary interstitial emphysema, pneumothorax, or grade 3 or grade 4 changes or more on the initial chest radiograph were more likely to be present in infants who died than in infants who survived (table 2). In addition, mean (SD) $PaCO_2$ was lower among survivors, being 4·1 (1·2), 4·5 (0·7), 4·8 (1·4), and 4·9 (1·1) kPa than among infants who died later, among whom the values were 5·7 (1·5), 5·7 (0·8), 7·6 (2·6), and 5·7 (1·7) at 6, 18, 36, and 72 hours, respectively. Similarly, in the second study mean (SD) $PaCO_2$ was lower among survivors, being 4·7 (0·8), 4·7 (1·0), 5·1 (0·7), and 5·3 (0·8) kPa compared with 4·9 (1·0), 5·7 (1·2), 6·3 (1·5), and 6·7 (1·9) at 6, 18, 36, and 72 hours, respectively. These observations suggest that differences in outcome were a result of variation in the severity of early lung disease (before the age of 72 hours), rather than more aggressive ventilator management resulting in more barotrauma among those that died later of respiratory causes.

Discussion

The results of this study suggest that in infants weighing less than 750 g at birth who survive the first 72 hours of life, ventilator requirements may predict late respiratory death from respiratory causes. The more pressure and oxygen required by very tiny babies the worse is the outcome; our findings support this impression but, in addition, we have identified and validated specific early predictors of later respiratory death. Using this method it has been possible to make a clear distinction between survivors and those who will die later. Our findings are dependent on the style of management, however, so should not be directly extrapolated to other intensive care nurseries. Although styles of management may differ elsewhere, our method of validating early predictors of late respiratory death could be applied in other centres.

It would be reasonable to expect treatment with surfactant to alter early peak inspiratory pressure requirements in infants weighing less than 750 g, so our findings cannot be extrapolated to infants being treated in this way.

Because follow up showed that survival until discharge corresponded with long term survival, survival until discharge was a valid end point to use in this study.

Our finding, that peak inspiratory pressure requirement was of more value than arterial: alveolar oxygen ratio in predicting late respiratory death, confirms those of Simbruner *et al.*[17] They found that early measurements of compliance predicted outcome in infants with respiratory distress syndrome. In addition, arterial:alveolar oxygen ratio is dependent on factors other than the pathology of the lung parenchyma, such as pulmonary perfusion and the shunting of blood between the pulmonary and systemic circulations.

In general, predictive information is useful to those managing infants who weigh less than 750 g at birth in the selection, or stratification by prognosis, of a study group in which it might be ethically justified to try new treatments. In making decisions of clinical management or in answering parents questions regarding individual patients, however, caution must be exercised. This is because we studied only a small number of infants in a single hospital. In addition, no comment can be made about infants whose peak inspiratory pressure requirement goes above then returns below the 95th centile, as no such cases were seen.

We would have expected the mean $PaCO_2$ to be higher in survivors than in those that died later of respiratory causes, at least during the early hours of life, if the different outcomes could be explained by more aggressive ventilator management of the latter infants. The reverse was the case, however, suggesting that the severity of lung disease is the main determinant of outcome. Radiological findings supported this supposition. As in previous studies, the presence of pulmonary interstitial emphysema in extremely low birthweight infants indicated a poor prognosis.[18]

In summary we have described a method for validating early predictors of late respiratory death in infants who weighed <750 g at birth. In our nursery, the peak inspiratory pressure requirement in the first 72 hours of life clearly distinguished survivors from those who died later. Peak inspiratory pressure requirement seemed to reflect the severity of the initial lung disease rather than the effect of ventilator management.

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Amniotic fluid insulin concentration as a predictor of obesity

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Abstract

Longitudinal correlations were obtained between amniotic fluid insulin concentration at 32 to 38 weeks' gestation and anthropometric characteristics at the age of 6 years in 56 children of diabetic mothers. The prospective studies indicated that at the age of 6 years, as at birth, the greatest increase in weight in relation to height (relative obesity) was seen in children who experienced the greatest exposures to insulin in the uterus (as judged by amniotic fluid insulin concentration). Significant correlations between amniotic fluid insulin and relative obesity at the age of 6 years were found after adjustment for maternal obesity and macrosomia at birth. The highest amniotic fluid insulin values are clustered in the subgroup of 14 children who were obviously obese by the age of 6 years. These findings are consistent with the hypothesis that there is an association between anthropometric development and intrauterine metabolism, and suggest that premature and excessive exposure to insulin during gestation may predispose to obesity in childhood. The amniotic fluid insulin concentration may predict this eventuality.

The possibility that maternal metabolism may exert an influence on the development of children was suggested by Freinkel a decade ago and designated as 'fuel-mediated teratogenesis'.[1 2] He proposed that maternal fuels may influence developmental events by modifying phenotypic gene expression in terminally differentiated, poorly replicating cells during intrauterine development; in addition, the long range effects will depend on which cells undergo differentiation, proliferation, or functional maturation (or a combination) at the time of the disturbance in maternal metabolism.[1 2]

The Diabetes in Pregnancy Center was established at Northwestern University Medical School in 1977 to test this hypothesis prospectively. Diabetes was chosen as the disease for study because maternal fuels undergo the greatest changes in this condition. Pregnant women with pregestational as well as gestational diabetes mellitus were enrolled during the period 1977–83. Their fuel economy was carefully monitored throughout pregnancy, and we have been following up their children since then in a prospective attempt to correlate their long range development with maternal metabolism during pregnancy. The present study reports some of the anthropometric consequences of intrauterine metabolic experiences ('fuel-related anthropometric teratogenesis'). We found significant correlations between pancreatic islet function of the fetus within the uterus and body shape in childhood. These conclusions are based on measurements in 56 children who now have reached the age of 6 years and in whom estimates of amniotic fluid insulin were obtained during weeks 32–38 of pregnancy. As far as we know this is the first direct evidence that exposure of a fetus to insulin may serve as a prognostic indicator of obesity in childhood.

Subjects and methods

Fifty six mothers were enrolled into this prospective study during the period 1977–83. The group was composed of 26 women with known diabetes mellitus who were receiving treatment before the index pregnancy (pregestational diabetes mellitus) and 30 with gestational diabetes mellitus. All the subjects with pregestational diabetes mellitus and 18 of 30 with gestational diabetes mellitus were treated with insulin given subcutaneously, as soluble insulin before each major meal (three times a day) and as intermediate acting insulin once or twice daily to sustain basal needs. Our objectives were to achieve 'tight' glycaemic control without frequent or severe attacks of hypoglycaemia, and to defer delivery until full term had been reached. Metabolic control was assessed from daily records of glucosuria and acetonuria, weekly or twice weekly measurements of fasting plasma glucose concentration, and monthly measurements of glycated haemoglobin (HbA_{1c}).

Amniotic fluid was sampled to monitor fetal lung maturation, and aliquots were frozen for later measurements of immunoreactive insulin concentrations.[3] Amniocentesis was carried out every two weeks starting at 32–34 weeks' gestation until full term. We found no association between insulin concentration and gestational age during this period. When two or more measurements were available the mean value was used. Anthropometric assessments were made of the infants at birth, and during subsequent follow up examinations by a variant of a measurement first proposed by Farquhar that we have designated the symmetry index.[4] On each occasion relative weight (that is, observed weight in relation to median weight for that age) to relative height (that is, observed height in relation to median height for that age) as follows:

$$\text{Symmetry index} = \frac{\text{observed weight/median for age}}{\text{observed height/median for age}}$$

According to this calculation a symmetry index of 1·0 indicates symmetrical adipose tissue and skeletal growth, whereas values of over 1·0 reflect asymmetry with relatively disproportionate increases in fat. On the basis of our experience with normal newborn infants and young children, symmetry indexes of over 1·2 indicate obesity. We have ascribed such asymmetrical relationships during periods of active growth to disproportionately greater development in structures that are most sensitive to the action of insulin than in those that are relatively insensitive.[1 5]

Analysis of variance and the Duncan method[6] were used to assess the significance of differences among the groups.

Results

Mean (SEM) gestational age at delivery in the 56 infants of diabetic mothers was 38·5 (0·2) weeks. At birth, their mean (SEM) weight was 3653 (87) g (range 2200–5580); length (height) 51·0 (0·4) cm (range 46·0–59·0); and symmetry index 1·17 (0·02) (range 0·88–1·53).

Mean estimates of immunoreactive insulin concentrations in amniotic fluid taken during weeks 32–38 of pregnancy correlated significantly with symmetry index at birth after controlling for maternal obesity by covariate analysis (r=0·362; p=0·003). The relationship was not, however, a simple linear one. Figures 1 and 2 show the antepartum amniotic fluid insulin concentrations and symmetry index values measured at the time of birth and at the age of 6 years in the 56 infants. Based on the symmetry index values these subjects were assigned to one of the three groups (group 1: symmetry index <1·0, group 2: symmetry index 1·0–1·2, and group 3: symmetry index >1·2). At birth (fig 1) seven (12%), 28 (50%), and 21 (38%) subjects were in groups 1, 2, and 3 respectively. There were significant differences among the groups (analysis of variance, p<0·05). Moreover, the amniotic fluid insulin concentration was significantly higher in group 3 (symmetry index >1·2) than group 2 (symmetry index 1·0–1·2), mean (SD) values being 125·5 (86·1) compared with 72·3 (62·8) pmol/l (p<·05).

At the age of 6, the height of the subjects did not differ from that expected for their age (observed height/median height for age=1·006 (0·039)). By contrast, mean weight was substantially increased (observed weight/median weight for age=1·137 (0·220)). The symmetry index for the whole group was 1·126 (0·185). When the infants were divided into three groups according to their symmetry index at the age of 6 years, a similar distribution of amniotic fluid insulin concentrations was observed (fig 2). A significant correlation between antepartum amniotic fluid insulin concentration and symmetry index values at the age of 6 years was seen in the entire group, even when we controlled for maternal obesity and symmetry index at birth as covariables (r=0·262; p=0·03). There were significant differences among the three groups (analysis of variance, p<0·01). On further analysis the highest amniotic fluid insulin concentrations that we had observed before birth were clustered predominantly and significantly in that subgroup of 14 infants (25%) with obvious obesity at the age of 6 years—that is, group 3 who had symmetry index values of over 1·2. Eight of these 14 infants had also had symmetry indexes of >1·2 at birth. On further analysis by the Duncan method,[6] antepartum amniotic fluid insulin concentrations in group 3 (symmetry index >1·2 at the age of 6) were significantly higher (p<0·05) than in the other two groups (140·5 (92·7), 86·1 (65·2), and 69·9 (56·8) pmol/l respectively).

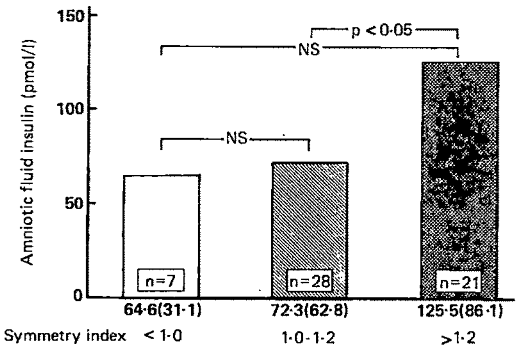

Figure 1 Association between symmetry index at birth in 56 children of diabetic mothers and amniotic fluid insulin concentrations during late gestation. The bars denote the mean amniotic fluid insulin concentration for each group; the values under each bar are the mean (SD). Significance of the differences between groups was determined by the Duncan method.[6]

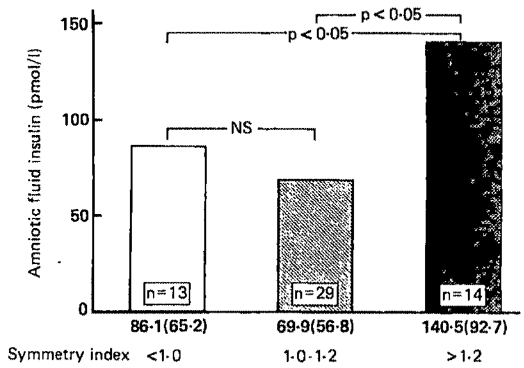

Figure 2 Association between symmetry index at age 6 and amniotic fluid insulin concentration during late gestation. The bars denote the mean amniotic fluid insulin concentration for each group; the values under each bar are mean (SD). Significance of the differences between groups was determined by the Duncan method.[6]

Discussion

During normal intrauterine life maternal insulin does not cross the placenta,[7 8] the secretion of fetal insulin is limited, and the functional responsiveness of fetal islets to nutrient secretogogues is tardy.[9–11] Hyperplasia, hypertrophy, and heightened functional reactivity supervene in fetal islets in pregnancies that are complicated by diabetes, presumably in response to the increased transplacental delivery of glucose[12] and other nutrient secretagogues.[1 13] Accordingly, late fetal development in an infant of a mother with diabetes may occur in a relatively insulin enriched environment. There are no easy ways of sampling fetal insulin production directly; because amniotic fluid insulin is wholly derived from fetal islets,[14] however, the concentration of insulin in amniotic fluid may act as an inferential index of integrated insulin secretion by the fetus, despite the uncertainties concerning rates of turnover and variations in the volume of amniotic fluid. We[5 15] and others[16 17] have previously shown that amniotic fluid insulin concentrations may be increased in even the mildest forms of gestational diabetes mellitus thus illustrating the exquisite sensitivity of fetal islet development to the stimulatory effects of ambient nutrients. As shown by symmetry index values at birth,[18] and patterns of intrauterine growth during serial ultrasonography,[5 19] the fetus of the diabetic mother seems to develop in an asymmetrical fashion whenever fetal insulin is increased so that insulin sensitive structures are stimulated to a disproportionate degree; this asymmetry seems to be characteristic of the macrosomia that has long been recognised in the offspring of diabetic mothers. The experiments of Susa *et al* with insulin minipumps implanted in the fetuses of non-diabetic, normoglycemic primates suggest that such asymmetrical development within the uterus can occur even without increases in the concentrations of circulating fuels and may be driven in a large part, by the premature, and perhaps inappropriate, availability of 'extra' insulin.[20]

The present findings assume particular importance in the above context. As judged by amniotic fluid insulin, we have shown that an increased availability of insulin to the fetus of the diabetic mother during intrauterine development may correlate not only with anthropometric characteristics at birth, but also with subsequent patterns of asymmetrical development—that is, the development of childhood obesity by the age of 6. Analysis of our follow up data indicates that this childhood obesity may be dissociated from maternal obesity and that it may supervene even when symmetry index values are less than 1·2 at birth. It is tempting to suggest that some of this propensity for asymmetrical growth and childhood obesity may be linked to the developmental impact of the accelerated islet maturation and enhanced insulin secretion during fetal life. The increases in amniotic fluid insulin could thus provide some direct augury for the long range consequences of such upsets of the developmental timetables ('fuel-mediated anthropometric teratogenesis').

While our prospective studies have been underway, Pettitt *et al* have reported their findings with the Pima Indians.[21] Review of the longitudinal data from this highly inbred population who are prone to non-insulin dependent diabetes mellitus has indicated that the infants of mothers who were diabetic during pregnancy already had a far greater incidence of obesity by the ages of 5–9 than those of mothers who became diabetic after the index pregnancy or remained normal, and that the distinctions were even more pronounced by the ages of 10–14 and 15–19.[21] Moreover, in the Pima Indians—as in the present series—the putative correlations between childhood or adolescent obesity and metabolic experiences during fetal life could also be disassociated from obesity in the mother, or the occurrence of macrosomia at birth, or both.[21 22] Unfortunately the available information concerning the state of maternal metabolism throughout pregnancy, and the attendant implications for the developing fetuses in the studies of Pima Indians, is limited to the single measurement of maternal plasma glucose that was made two hours after a glucose load had been given to classify gestational glucose tolerance. None the less, these important observations also implicate the maternal environment as a contributor to subsequent obesity and are consistent with the hypothesis of 'fuel-mediated anthropometric teratogenesis'.[1 2]

Our prospective study has shown that amniotic fluid insulin concentration as an index of prematurely activated fetal insulin secretion may be a prognostic indicator of obesity in childhood. Whether the premature exposure of the fetus to insulin affects subsequent anthropometrics by phenotypic modifications of cells concerned in postnatal sensitivity to insulin in the periphery, insulin secretion, or neuroendocrine factors that govern energy expenditure or fuel intake, or counterregulation, or a combination, are questions that we are investigating in our continuing studies.

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Norbert Freinkel died on 5 September 1989.

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Ramadan and birth weight at full term in Asian Moslem pregnant women in Birmingham

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Abstract

The birth weights of 13 351 babies born at full term from 1964–84 to Asian Moslem mothers in Birmingham were analysed to see if the effect of the Ramadan fast on maternal biochemical profiles was of any clinical relevance. These were compared with two age matched control groups comprising white and non-Moslem Asian babies. Ramadan had no effect on mean birth weight at whatever stage of pregnancy it occurred. There was an increase in the prevalence of low birth weights (4·5% to 8%) among babies who were born at full term when Ramadan had occurred during the second trimester, but this was not significant.

We conclude that the Ramadan fast has no effect on the birth weights of babies born at full term.

Moslems everywhere are required to fast from sunrise to sunset during the month of Ramadan. This is the ninth month of the Islamic calendar and begins 11 or 12 days earlier each successive Gregorian year.[1] Certain Moslems are given dispensation, including women who are pregnant. Most choose to fast at the set time, however, because all missed days must be repaid later, and it is easier to fast as a group than alone.[2 3]

Various metabolic changes occur in healthy adults taking part in such a fast. Mustafa *et al* found increases in serum uric acid and lipid concentrations as well as changes in overall fluid balance.[4 5] Furthermore, nutritional studies have shown that with a reduction in the frequency of meals food intakes are generally lower, with overall reduction in body weight,[6] more significant in women.[7] Similar metabolic changes have been described in pregnant women who observe the Ramadan fast in Gambia[3] and Birmingham,[8] and these included reduced blood glucose concentrations and raised plasma concentrations of free fatty acids and ketones. The clinical importance of these metabolic changes and in particular their effect on the outcome of pregnancy is, however, unclear.

The aim of this study was to determine whether Ramadan affected one clinical outcome of pregnancy—that is, birth weight.

Subjects and methods

Data were drawn from the Birmingham births data from 1964 to 1984.[9] The race of the mother is included in the data set, but categorises Asians as a single group. For this study it was necessary to distinguish between Moslem and non-Moslem (Sikh and Hindu) births. Preliminary work was therefore undertaken in order to separate these groups.

DISTINCTION BETWEEN MOSLEM AND NON-MOSLEM ASIANS

The births data set includes the first three letters of the mother's maiden surname. Using these letters Asian births were allocated to three groups ('Moslem', 'non-Moslem', and 'uncertain'). In a small subgroup, the antenatal records of 100 mothers were reviewed at Sorrento Maternity Hospital. With the aid of literature on Asian names[10–12] it was possible to determine certain three letter prefixes that could be allocated to Moslem and non-Moslem surnames. This categorisation was then tried on a subset of the Birmingham data 1964–78 (19 111 Asian births). It was realised at this stage that some births would have to be omitted from the study because of the overlap of certain prefixes (for example, Kau may be Kauser (Moslem) or Kaur (Sikh)); 37% of Asian names could be allocated to the Moslem group with certainty, 49% were non-Moslem, and 14% could not be allocated. Having decided which three letter prefixes were in which categories, the same procedure was used for distinguishing between Moslem and non-Moslem Asians in the main data set.

MAIN STUDY

For each Asian Moslem birth, a white and a non-Moslem Asian birth that had taken place within two days of the Moslem birth were selected. This controlled for secular and seasonal changes in distribution of birth weights. It was not always possible to select a non-Moslem Asian control because there was not always a non-Moslem Asian birth within two days of a Moslem birth. The numbers in this group are therefore smaller. We selected 13 351 Asian Moslem, 13 351 white, and 5 106 non-Moslem Asian births by this method. Only babies born at full term (38 to 41 weeks inclusive, the data set available) were included. The date of Ramadan in relation to the birth was then calculated.

Two items were used as measures of final outcome, the mean birth weight and the proportion of low birthweight babies. In order to look at sequential four week periods (Ramadan lasts for four weeks) the four week moving mean birth weight was calculated. A similar procedure was followed for proportions of low birthweight

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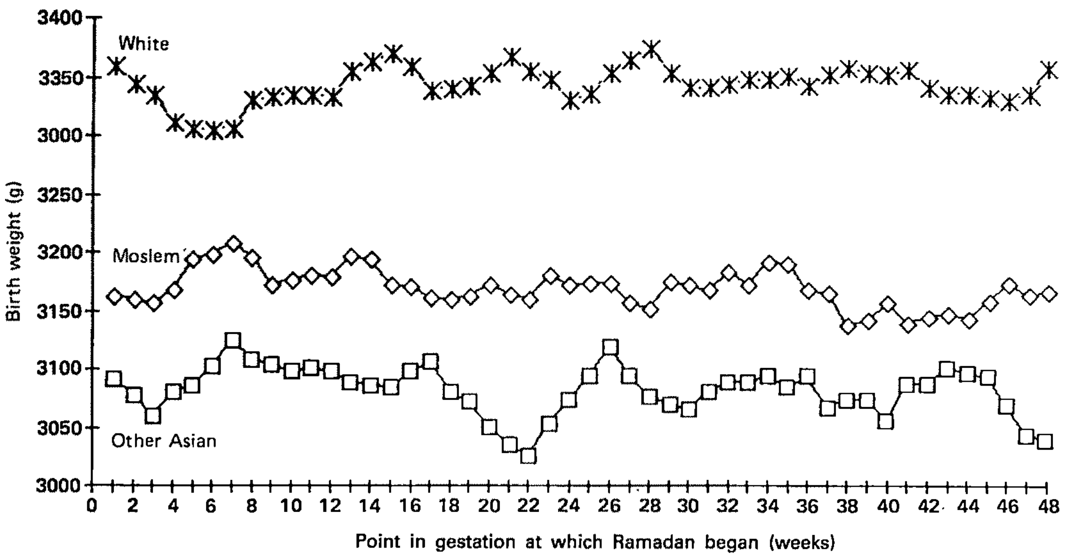

Figure 1 The effect of Ramadan on moving mean birth weight, 1964–84.

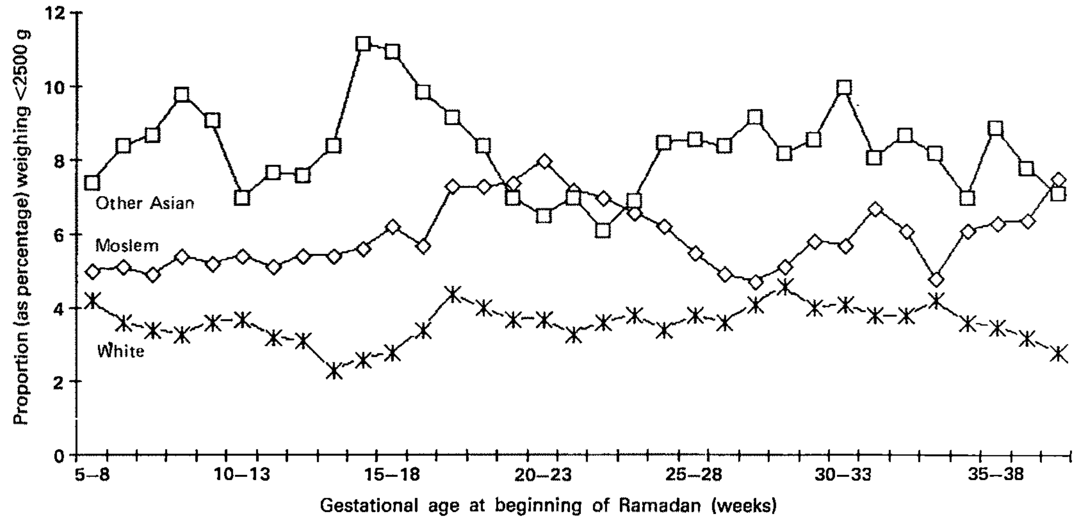

Figure 2 The moving four week proportion of low birthweight babies and Ramadan (plot of mean proportion for each consecutive four week period).

infants. In order to study further the effect of Ramadan, the data were reviewed in five year periods. This would show—for example—if there had been a secular trend in the observance of Ramadan, and therefore its effect on birth weight.

Results

CUMULATIVE ANALYSIS

Figure 1 shows the four week moving mean birth weight in relation to the timing of Ramadan for the three groups. There were no discernible changes in mean birth weight in relation to Ramadan in any of the groups. What is clearly shown, however, is that the mean birth weight of white infants was greater than that of Moslems (mainly Pakistani), which was in turn greater than that of non-Moslem Asians (mainly Hindus), confirming previous reports.[13 14]

Figure 2 shows the moving four week proportion of low birth weight in relation to the timing of Ramadan during gestation. There is a peak in the number of low birthweight babies delivered to Moslem mothers when Ramadan began at 17 to 25 weeks' gestation, and a similar peak in non-Moslem Asian births when Ramadan started during the period 13 to 18 weeks' gestation. Neither of these peaks was significant using a test of heterogeneity.[15]

QUINQUENNIAL ANALSIS

Figure 3 shows the moving mean birth weight for each five year period from 1964 to 1984 and where Ramadan occurred during gestation, among Moslems. If Ramadan had an effect on birth weight, a depression would have been seen in each line at the same point in the plot for each five year period. The depression would, however, move as Ramadan moved if the effect was seasonal. No such depressions are seen, but there was a general overall increase in birth weight over the 20 year period.

Figure 4 shows a similar quinquennial analysis of the moving proportion of low birthweight infants and where Ramadan occurred during gestation. In this instance if Ramadan had an effect there would be peaks in each graph, each occurring at a similar point. Similarly if there

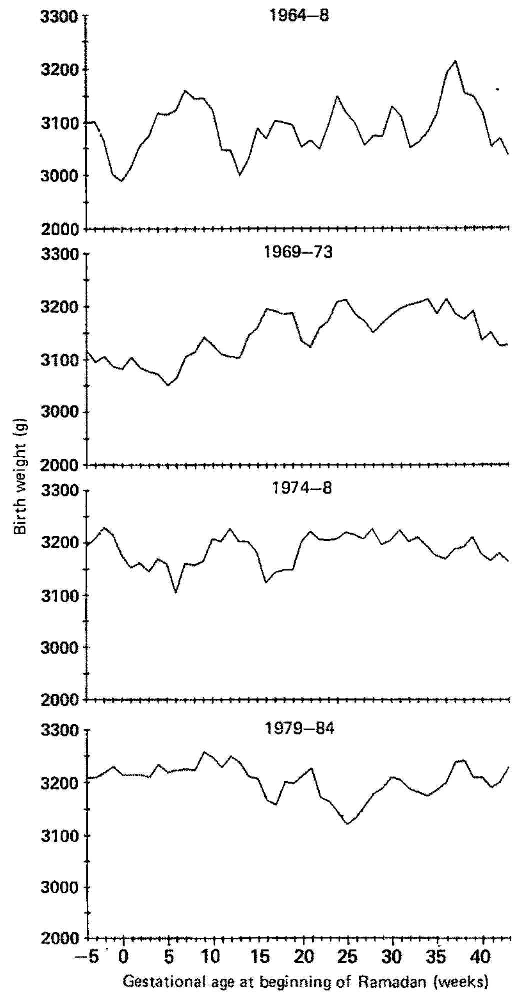

Figure 3 Moving mean birth weight and Ramadan (Moslem).

was a seasonal effect the peaks would move as Ramadan moved. A consistent peak is suggested on three of the four graphs (1969 to 1973, 1974 to 1978, and 1979 to 1984); this occurred when Ramadan started during weeks 17 to 24. There was no signficance, however, when a test of heterogeneity was used.[15]

Discussion

Using available data on births in Birmingham between the years 1964 and 1984 neither visual trends nor statistical analysis suggested any effect of Ramadan on mean birth weight. Selection of data was dependent on tracing religion by prediction of names using three letter prefixes. There may have been some inaccuracies in this and some prefixes could not be allocated with certainty. This bias is unlikely to have had a systematic effect, however, and any random effect would have decreased the differences between the groups rather than exaggerated them. We have no indication of how many of the women included in the study adhered to Ramadan during pregnancy, although from the results of other studies at this hospital it seems that most Moslem mothers do observe the fast.[2 8]

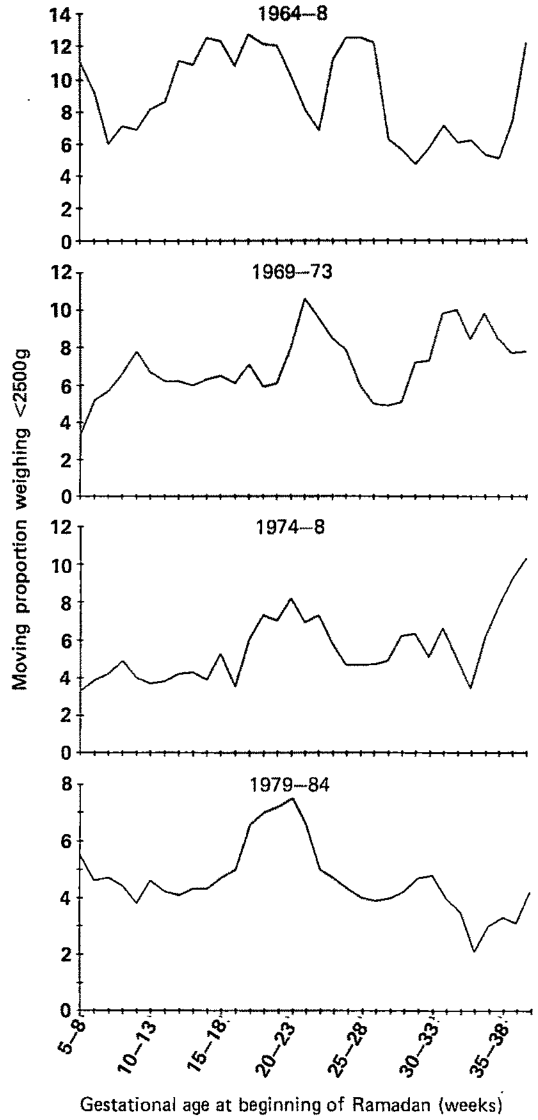

Figure 4 Quinquennial moving proportion low birth weight and Ramadan (Moslem) (plot of mean proportion for each consecutive four week period).

The effect of Ramadan on the prevalence of low birth weight was less clear cut. Both the cumulative and three of the four periods of the quinquennial analysis suggested that when Ramadan began in the middle trimester there was an increase in the proportion of low birthweight babies subsequently born at full term. During the second trimester mothers lay down stores of fat in anticipation of later fetal demands,[16] and failure to do so is associated with lower birth weight among Asian mothers.[17 18] It was tempting to speculate that the metabolic disturbances of Ramadan fasting at this time had interfered with fetal growth. In clinical terms these variations in prevalence of low birth weight were quite pronounced, being from 4·5% to 8%, whereas in a similarly large number of white births, variation during the year was less (2·5% to 4%). These visual impressions were, however, not significant. In view of the large number of births it seems unlikely that this can be explained by a type II error. We therefore conclude that Ramadan has no effect on the prevalence of low birth weight in babies born at full term.

We are grateful to Professor EG Knox and the department of social medicine, Birmingham Medical School for permission to use the data, and to Mr Robert Lancashire for extracting the data and performing calculations.

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Quantitative changes in faecal microflora preceding necrotising enterocolitis in premature neonates

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Abstract

Quantitative studies of faecal bacterial flora were carried out during the week preceding the clinical onset of 12 episodes of neonatal necrotising enterocolitis. There were considerable quantitative changes in the faecal flora preceding the clinical onset of both definite and possible episodes of necrotising enterocolitis. There was a decline in the numbers of some species from up to 72 hours before the clinical onset of the disease. Enterobacteriaceae were isolated from samples collected during the 48 hours preceding the clinical onset of all four definite episodes of necrotising enterocolitis. These were 'new' isolates in two episodes, and considerably increased numbers in another.

The changes that we found are probably the result of changes in intraluminal conditions that precede the clinical onset of necrotising enterocolitis.

The aetiology of neonatal necrotising enterocolitis remains unknown; its incidence rises with increasing prematurity and may reach over 13% in very low birthweight infants.[1] There may be temporal and geographical clustering of cases, suggesting that a transmissible agent may play a part.[2] Previous studies of faecal flora associated with neonatal necrotising enterocolitis have concentrated on the speciation of the microbial flora present at the onset of clinical signs of disease. *Clostridium* spp,[3 4] enterobacteriaceae,[5–7] and—most recently—coagulase negative staphylococci[8] have been associated with the disease. Studies of faecal α_1-antitrypsin[9] and breath hydrogen concentration,[10] however, together with histological evidence (W Ballance, B Dahur, N Sheuher, R Kliegman. Proceedings of a Symposium 'Neonatal necrotising enterocolitis: current concepts and controversies'. Columbus, USA, 1989) suggest that the pathological process leading to necrotising enterocolitis may precede the onset of clinical signs of disease by hours or even days. The bacterial species present at the clinical onset therefore may not be representative of those present at the onset of the pathological process.

We describe quantitative changes in faecal microflora in the weeks preceding the onset of 12 episodes of neonatal necrotising enterocolitis.

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Materials and methods

We studied the faecal flora of premature neonates with episodes of illness associated with specific signs of gastrointestinal dysfunction including abdominal distension, blood in the stools, gastric retention, abdominal wall erythema, and abnormal abdominal radiographs. Radiological abnormalities were evidence of ileus, thickening of the bowel wall, or 'rigid' loops of bowel. Episodes were divided into those associated with intramural or intrahepatic gas (definite) and those without such gas (possible).

SAMPLE COLLECTION AND STORAGE

Sample of faeces (when available) were collected by nursing staff each day from all neonates of less than 32 weeks' gestational age who had been admitted to the Peter Congdon Regional Neonatal Unit with respiratory distress syndrome between February and September 1989. Approximately 0·2 g of faeces was inoculated into a phial containing 1·8 ml of prereduced glycerol citrate broth[11] in an anaerobic cabinet (Wise Anaerobic Work Station, Don Whitley, Shipley). The weight of the sample was determined by subtracting the known weight of the phial from the weight after the addition of the sample. Phials containing faecal samples were stored at −70°C. Dry weight as a percentage of wet weight was determined after drying a weighed sample in a hot air oven for 24 hours.

DETERMINATION OF QUANTITATIVE BACTERIOLOGY

Quantitative bacteriology was carried out within eight weeks of storage of the samples at −70°C. Samples were thawed at room temperature and 100 µl of the contents of the phial were serially diluted tenfold in brain heart infusion broth (Oxoid CM 225). From each dilution 100 µl was inoculated onto the following solid media and incubated under the following conditions: blood agar base (Oxoid CM 55) containing 5% horse blood incubated at 37°C in air; heated blood agar at 37°C in 5% carbon dioxide; MacConkey agar without salt (Oxoid CM 7b) at 37°C in air; Sabouraud's agar (Oxoid CM 41) with chloramphenicol 50 mg/l at 30°C in air; VCAT agar (GC agar base (Oxoid CM 367) containing 10% lysed horse blood, GC supplement (Oxoid SR 105), vancomycin 5 mg/l, colistin 6 mg/l, amphotericin B 1 mg/l, and trimethoprim 5 mg/l) at 43°C under microaerophilic conditions; and Slanetz-Bartley agar (Oxoid CM 377) at 37°C in 5% carbon dioxide. The following solid media were inoculated and incubated at 37°C under anaerobic conditions (Wise Anaerobic Work Station): Columbia blood agar base (Oxoid CM 331) with 5% horse blood; Rogosa's agar (Oxoid CM 627);

Wilkins-Chalgren agar (Oxoid CM 619) with 5% horse blood, sodium pyruvate 1 g/l, and nalidixic acid 10 mg/l; Veillonella agar (Difco Ltd); Columbia blood agar with kanamycin 100 mg/l and vancomycin 7·5 mg/l; and fructose agar (*Clostridium difficile*) (Oxoid CM 601) with 7% horse blood, cycloserine 500 mg/l, and cefoxitin 16 mg/l. Media incubated under anaerobic conditions were supplemented with hemin, menandione, and sodium bicarbonate. In addition, for the isolation of clostridia, 100 µl of the contents of the phial were added to 100 µl of 95% ethanol.[12] This mixture was incubated for 30 minutes at 37°C before inoculation on to egg yolk agar,[12] prepared with blood agar base (Oxoid CM 55), which was then incubated at 37°C under anaerobic conditions. All agar plates were examined for growth for up to one week after inoculation. Colonies were counted using an automated colony counter. Isolates were identified using standard laboratory methods.[12]

'New' isolates were defined as those strains isolated in the 48 hours preceding the clinical onset of necrotising enterocolitis but not from the samples collected in the five days before that time.

Results

A total of 752 stool samples were obtained from 90 neonates during the eight month period of the study. Seven definite episodes of necrotising enterocolitis and 11 possible episodes were diagnosed. Faecal samples were obtained during the week preceding the clinical onset of four (four babies) of the seven definite episodes and eight (seven babies) of the 11 possible episodes. The neonates who developed possible necrotising enterocolitis and from whom samples were available had a median gestational age of 27 weeks (range 26–30) and median birth weight of 995 g (range 660–1160). Neonates who developed definite necrotising enterocolitis and from whom samples were available had a median gestational age of 28 weeks (range 27–30) and median birth weight of 725 g (range 620–1440). Antibiotics were given intravenously to the babies in the week before three of the eight episodes of possible and one of the four episodes of definite necrotising enterocolitis.

All the episodes of possible and definite necrotising enterocolitis followed the introduction of nasogastric feeding (by intervals ranging from three to 16 days). In the intensive care unit there is a trend towards an increase in numbers of strains present in the faeces with time after the introduction of nasogastric feeds. A sudden decline in numbers of facultative species such as *Streptococcus faecalis* or *Staphylococcus* spp is unusual, unless it is associated with antibiotic treatment. The quantitative changes in flora before three definite episodes of necrotising enterocolitis (in three neonates) are shown in figs 1–3. Samples were available only from the 48 hours before the onset of the fourth definite episode of necrotising enterocolitis; a strain of *Klebsiella pneumoniae* was isolated at a concentration in excess of 10^{11} colony forming units (cfu)/g dry weight in the 48 hours before this fourth episode. Bacterial strains from the genus enterobacteriaceae were isolated from samples collected in the 48 hour periods before the onset of all four definite episodes of necrotising enterocolitis. The species involved were *Escherichia coli*, *K pneumoniae* (two episodes) and *Enterobacter cloacae*. Two of these strains were 'new' isolates (figs 1 and 3) and in a third episode (fig 2), a strain of *E cloacae* increased considerably in concentration before the onset of necrotising enterocolitis. There

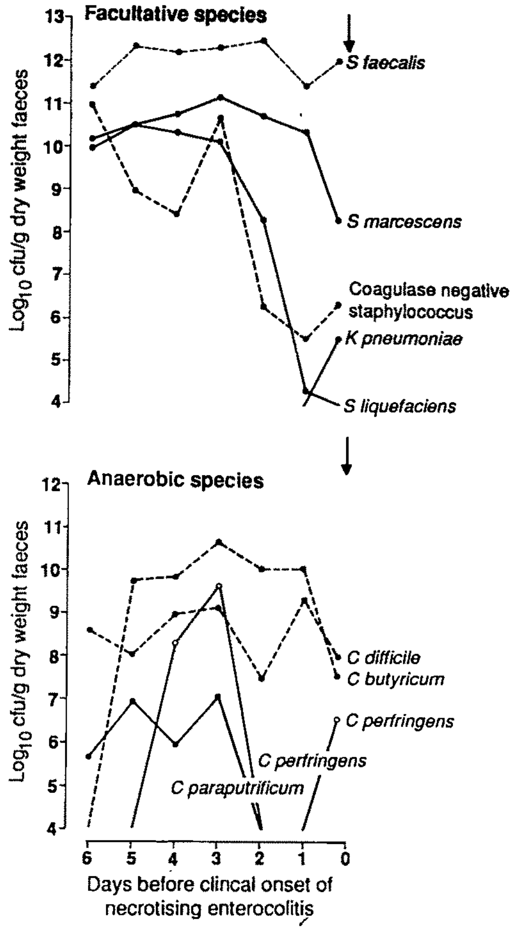

Figure 1 Changes in facultative and anaerobic species of faecal flora during the week before the clinical onset of neonatal necrotising enterocolitis.

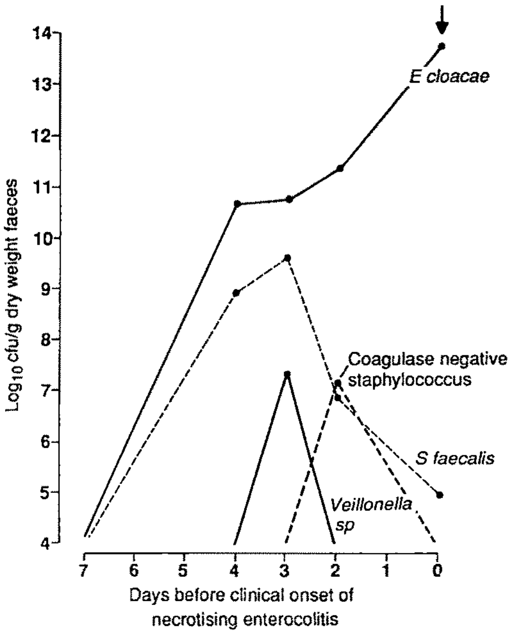

Figure 2 Changes in numbers of E cloacae, S faecalis, coagulase negative staphylococci, and Veillonella sp during the week before the clinical onset of neonatal necrotising enterocolitis.

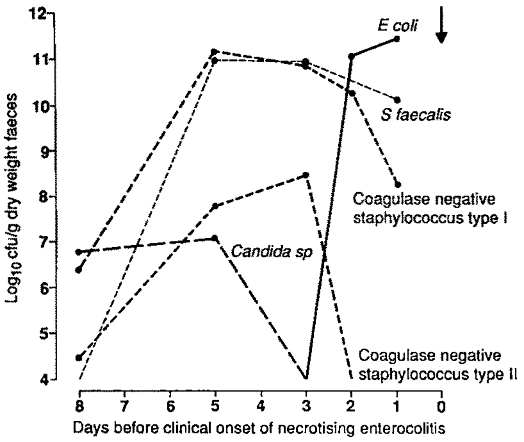

Figure 3 Changes in numbers of E coli, S faecalis, coagulase negative staphylococci types I and II, and Candida sp during the week before the clinical onset of neonatal necrotising enterocolitis.

was a quantitative decline in the concentration of other bacterial species including both facultative and anaerobic species from up to 72 hours before all three definite episodes of necrotising enterocolitis.

In the week before the onset of one of the definite episodes of necrotising enterocolitis (fig 1) four species of clostridia were isolated; this was an unusual pattern of colonisation. Although *Clostridium* spp were isolated from 19 of 121 samples (nine neonates) collected from 29 neonates admitted to the unit between six weeks before, and six weeks after, the onset of this episode of necrotising enterocolitis, in only three samples from one neonate were two species of clostridia isolated. There were no samples with three or more species.

Four of the eight possible episodes of necrotising enterocolitis were not preceded by quantitative changes (rise or fall of two or more microbial species by twice $\log_{10}$ cfu/g dry weight) or by the detection of 'new' isolates. 'New' isolates associated with the other four possible episodes of necrotising enterocolitis were *Serratia marcescens* and *S faecalis* (episode 1); *S faecalis, Clostridium perfringens,* and *Veillonella* spp (episode 2); *S faecalis* (episode 3); and *Veillonella* spp, *Propionibacterium* spp, *Candida* spp, and *E cloacae* (episode 4).

Discussion

The intramural (or intrahepatic) gas that is characteristic of necrotising enterocolitis is probably a result of bacterial fermentation of intraluminal substrates,[13] and bacterial fermentation of carbohydrates in the small intestine before the clinical onset of the disease.[10] Clark *et al* reported that mucosal lesions could be induced in the rabbit ileum by the intraluminal injection of acidified casein, and suggested that the reduction in pH resulting from bacterial fermentation of excess substrate within the small bowel of neonates, particularly by enterobacteriaceae that ferment lactose rapidly,[14 15] may lead to similar conditions to those that induce lesions in the rabbit model of necrotising enterocolitis.

The concentration of breath hydrogen has been positively correlated with the numbers of enterobacteriaceae in the faeces of neonates.[16] Previous studies have suggested a link between enterobacteriaceae and necrotising enterocolitis,[5–7 17–20] but no one species has consistently been linked with it. In this study, enterobacteriaceae were isolated from samples collected during the 48 hours before the clinical onset of all four definite episodes of necrotising enterocolitis. These were 'new' isolates in two episodes and a considerable increase in numbers in another episode. By contrast with episodes of possible necrotising enterocolitis, new isolates of other genera were not found in the 48 hours before the clinical onset of the definite episodes. Enterobacteriaceae were isolated from samples preceding two of the eight possible episodes of necrotising enterocolitis, and were isolated in association with 'new' isolates from other genera in those episodes. The decline in numbers of some bacterial species in the faecal flora probably resulted from alterations in substrate availability and pH after bacterial fermentation in the small intestine.

Bacterial fermentation in the small intestine may contribute directly to the pathogenesis of necrotising enterocolitis,[14 15] or it may result from changes in intraluminal conditions arising as a result of the disease. The changes that we have described probably result from changes in intraluminal conditions that precede the clinical onset of necrotising enterocolitis.

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Outcome after acute osteomyelitis in preterm infants

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Abstract

Eight cases of skeletal infection in preterm infants were studied. All the infants were systemically unwell, with polymorpholeucocytosis. Diagnosis was by blood culture, and any radiographic changes were apparent at the time of presentation. Infection was often multifocal, with sites around the knee being most commonly affected. *Staphylococcus aureus* was the pathogen isolated in six of the eight cases; in these treatment with fusidic acid was effective and well tolerated, even at doses that were less than the recommended therapeutic minimum.

Even with prompt diagnosis and aggressive treatment orthopaedic sequelae are common.

Acute osteomyelitis is an uncommon but important neonatal infection. In this paper we report the methods of treatment and results in eight preterm patients with acute osteomyelitis.

Patients and methods

Eight preterm infants with skeletal infections were admitted to our neonatal intensive care unit between 1982 and 1988; their salient clinical features are summarised in the table.

All eight children were preterm, being born between 25 and 33 (mean 29·4) weeks' gestation. All were admitted to the neonatal unit for treatment of the consequences of prematurity.

Four of the eight had umbilical arterial lines in place at the time of the development of the septic episode. In each patient sepsis was suspected on clinical grounds before the presentation of any localising signs. All eight patients had considerably increased white cell counts. In all eight the clinical presence of infection prompted the performance of a 'septic screen'. Organisms were isolated from the primary blood cultures in each patient and in addition three patients grew organisms from other sites—the umbilical stump, cerebrospinal fluid, and an infected intravenous cannula. The pathogens isolated were *Staphylococcus aureus* in six of the eight patients, and group G β haemolytic streptococcus and *Candida albicans* in the remaining two.

There was a delay (mean 2·9 days, range 0–8)—especially early in the series—between presentation with systemic signs and the definitive diagnosis of skeletal sepsis. In the most recent four patients the definitive diagnosis was made on the day of presentation.

Five of the eight patients were treated by some form of drainage procedure (aspiration, n=3; open surgical drainage, n=2). All patients were treated with sodium fusidate, 20 mg/kg/day, initially intravenously (for a mean of 11 days, range 7–21), and orally when they were tolerating oral feeds (for a mean of 22 days, range 21–27). Treatment lasted for a mean of 32 days (range 28–42). An additional antistaphylococcal antibiotic was used when appropriate. Initially serum concentrations of fusidic acid were measured by high performance liquid chromatography. Despite clinical improvement in all eight patients, therapeutic concentrations of fusidic acid were not achieved with either oral or parenteral treatment. Serum bactericidal titres were also below the recommended therapeutic minimum. The duration of treatment was determined by each infant's clinical progress, and in one patient it continued after discharge from hospital.

Six patients were reviewed in the follow up clinic. The recent progress of the two non-attenders was obtained from their general practitioners.

Results

The patients had been followed up for between 2 years 2 months and 6 years since their infections. Two of the eight children failed to attend the specially arranged follow up clinic, but had normal musculoskeletal examinations when last seen in the orthopaedic clinic at 3·5 years of age.

Skeletal sepsis seemed to have been abolished by treatment in all patients. At the time of follow up no patient had developed chronic osteomyelitis.

In six of the eight patients the radiographic appearance was diagnostic of skeletal infection. The presence of radiographic changes did not seem to be related to the length of time that sepsis had been present, as there were changes on the radiographs of patients taken on the day that they had presented with systemic upsets. Two patients had normal radiographs throughout, and in two (one of whom had a diagnostic radiograph) ^{99m}Tc labelled diphosphonate scintigraphy was carried out; both scintigrams were normal.

Long term orthopaedic sequelae, such as discrepancies in limb length, angular deformity, and joint stiffness developed in five of the eight patients. Progressive discrepancy in limb length was the commonest long term problem (n=5), with joint destruction and axial deformity each occurring in one patient. One patient (case 7) has had his deformity corrected surgically. Limb lengthening is planned for the same patient in the near future.

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Clinical details of eight neonates with acute osteomyelitis

Case No	*Birth weight (g)*	*Gestation (weeks)*	*Age at presentation*	*Clinical features*	*Bacteriology*	*Treatment*	*Outcome*
1	1230	29	Day 5 systemic upset: blood culture Day 11 left distal femur Day 13 right distal femur Day 19 left distal tibia	Apnoea, Swollen knee White cell count $22\,000\times10^9$/l Radiograph normal Scintigram normal	*S aureus* in blood and aspirate	Aspiration; fusidic acid and erythromicin	Full movements; 2 cm shortening at 6 years of age
2	1800	32	Day 10 systemic upset: blood culture Day 13 left proximal femur	White cell count $25\,000\times10^9$/l Radiograph hip: dislocation, gross osteomyelitis	*S aureus* in blood and aspirate	Surgical drainage; fusidic acid and erythromicin	Decreased movement; 3 cm shortening at 2·2 years of age
3	1790	31	Day 7 systemic upset: blood culture Day 9 right distal femur	White cell count $45\,000\times10^9$/l Radiograph: osteomyelitis	*S aureus* in blood and aspirate	Aspiration; fusidic acid and flucloxacillin	No sequelae at 3 years of age
4	1800	33	Day 7 systemic upset: cerebrospinal fluid and blood culture Day 19 right distal femur Day 21 right proximal femur Day 21 left proximal tibia	White cell count $29\,000\times10^9$/l Radiograph: osteomyelitis Scintigram normal	*S aureus* in blood and cerebrospinal fluid	No drainage; flucloxacillin and chloramphenicol, followed by fusidic acid	No sequelae at 3·5 years of age
5	1560	31	Day 15 systemic upset: blood culture Day 15 right distal femur Day 17 left proximal tibia	Hot swollen knee White cell count $29\,000\times10^9$/l Radiograph normal	*S aureus* in blood and aspirate	Surgical drainage; fusidic acid and erythromicin	No sequelae at 3·5 years of age
6	620	25	Day 7 systemic upset: blood culture left proximal femur	Abdominal distension White cell count $30\,000\times10^9$/l Radiograph: osteomyelitis	*S aureus* in blood and aspirate	Aspiration; fusidic acid and flucloxacillin	Full movement; 1 cm shortening at 3·5 years of age
7	800	26	Day 19 umbilical line Day 49 left distal femur	White cell count $25\,000\times10^9$/l Radiograph: osteomyelitis	*C albicans* in blood and catheter tip	No drainage; amphotericin and cefuroxime	30° Flexion deformity at knee; 8 cm shortening at 6·5 years of age
8	1100	28	Day 10 skin abscess Day 16 right distal femur Day 16 right proximal femur	White cell count $23\,000\times10^9$/l Radiograph: osteomyelitis	β haemolytic streptococcus in pus from skin abscess, and from blood	No drainage; fusidic acid and penicillin	No sequelae at 3·5 years of age

Case 7 had a candida infection of his umbilical line on day 16 that persisted. He became 'septic' on day 49 with a candida septicaemia and osteomyelitis.
Case 8 had a skin infection on day 10, but did not become systemically unwell until day 16, when she had localising signs of skeletal sepsis.

The orthopaedic outcome was not related to the severity of initial infection, delay in making a definitive diagnosis, or the performance of surgical drainage.

Discussion

Osteomyelitis is an uncommon but important neonatal infection with a recognised morbidity and mortality.[1] The definitive treatment and its duration has not yet been defined.

Osteomyelitis in well nourished infants born at full term seems to cause little in the way of systemic upset,[2 3] but constitutional symptoms were much more of a feature in a series of Nigerian infants born at full term.[1] Two of the four patients with osteomyelitis associated with umbilical catheter sepsis reported by Brill *et al* were systemically unwell,[4] but two had no generalised features of sepsis. Only one of their babies who had systemic symptoms was preterm. All eight of our patients were preterm and all had systemic symptoms. We suggest that osteomyelitis in infants who are immunocompromised because of malnourishment or prematurity is much more likely to be accompanied by septicaemia and systemic upset. Multifocal disease occurred in four of our patients and this accords with the experience of other authors.[5]

Blood cultures from all our patients grew pathogens, and were helpful in deciding definitive antimicrobial treatment. When drainage procedures were carried out they served both to decompress and to aid diagnosis. Identical organisms were grown from the aspirate and the blood cultures. All patients treated without operation made good recoveries from their systemic upsets once treatment with antibiotics was started.

Other authors have reported the occurrence of bone and joint infection distal and ipsilateral to the tip of umbilical artery catheters, especially if vascular spasm accompanied its introduction.[6] It was suggested that osteomyelitis associated with umbilical catheterisation may be avoided by adherence to a protocol incorporating strict asepsis and immediate removal of the catheter if there was vascular compromise. In our patients who had umbilical arterial catheterisation, the catheter tip was at L4/5, and there was no suggestion of vascular insufficiency. The distribution of the affected joints was not significantly different from that in the patients without umbilical catheters, and we do not think that umbilical arterial catheterisation was an important factor in the development of sepsis.

The presence of radiographic signs at presentation in neonates has not been noted previously to our knowledge, and may suggest that the infection had been present subclinically before presentation. The limited value of skeletal scintigraphy was commented on by Ash and Gilday.[7]

The effectiveness and lack of serious side effects of fusidic acid in the treatment of staphylococcal neonatal osteomyelitis is well documented.[8 9] The use of a second antistaphylococcal drug is essential to prevent the emergence of resistant strains.[10] The fact that adult therapeutic concentrations or recommended bactericidal concentrations were never achieved despite considerable clinical improvement is interesting, and illustrates the difference between the in vivo and in vitro effects of drugs. It is a matter of orthopaedic tradition and dogma that long courses of antibiotic (usually six weeks) are used for skeletal infection. This does not seem to be necessary in preterm infants.

The distinction between septic arthritis and osteomyelitis is often difficult in infants. The epiphyseal circulation is derived from the same periarticular anastamoses as the metaphyseal circulation and this common circulation provides a pathway for the transphyseal spread of infection. It would therefore seem likely that the primary skeletal infection is metaphyseal osteomyelitis, but a secondary septic arthritis soon develops. The growth plate is surrounded by infected tissue and its blood supply, which is almost exclusively from the metaphyseal circulation, is compromised.

Most of the orthopaedic complications were the results of physeal damage by the septic process, with progressive discrepancy in limb length being caused by symmetrical physeal damage, and axial deformity being caused by asymmetrical physeal damage.

CONCLUSIONS

In preterm infants the clinical picture of acute osteomyelitis is different from that in babies born at full term and in older children, in that systemic upset and pronounced polymorpholeucocytosis are invariably present. Any radiographic changes were apparent at the time of presentation.

It is important to keep the possibility of skeletal sepsis in mind, and any child with a clinical diagnosis of sepsis, especially staphylococcal septicaemia without an obvious focus, should have the hips and knees carefully examined and radiographed.

Treatment with sodium fusidate and a second antistaphylococcal drug, initially given intravenously, achieved satisfactory antimicrobial effect, although bony sequelae were common.

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Skin conductance and arousal in the newborn

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Abstract

We measured skin conductance continuously from the sole of a foot in babies of different conceptional ages before, during, and for 10 minutes after a 'heel prick' carried out for routine blood sampling. We studied 82 healthy babies whose gestational and postnatal ages ranged from 25–42 weeks, and 1–73 days.

The median skin conductance level (preheel prick) in babies of 40–43 weeks' conceptional age was 0·6 µS (microsiemens) and differed significantly between awake babies (1·2 µS) and those who were asleep (0·5 µS). In contrast babies less than 40 weeks had a significantly lower median skin conductive level (0·3 µS) which was identical in awake and asleep babies.

In response to the heel prick all babies became aroused and skin conductance rose sharply and immediately in 21 out of 22 (95%) babies 40–43 weeks' conceptional age, and in seven out of 23 (30%) babies 36–39 weeks. The median rise at one minute in babies of 40–43 weeks was significantly higher than those 36–39 weeks (2·7 µS compared with 0·5 µS). No babies less than 36 weeks had a change in their skin conductance after the heel prick. These results are consistent with the notion that 'emotional sweating' is a function of maturity and does not develop until 36 weeks' conceptional age.

When a small electrical current at constant voltage flows between two electrodes attached to the skin the amount of current passed is proportional to the skin conductance level. The activity of the eccrine sweat glands, controlled by postganglionic cholinergic fibres of the sympathetic nervous system, is one important factor that influences the skin conductance level. The plantar and palmar sweat glands are activated by emotional arousal. In adults measurements of electrodermal activity from electrodes at these sites have been used by psychologists as a marker of arousal,[1] by psychiatrists as a biological approach to the study of many psychiatric disorders,[2] and by criminologists as a marker of deception.[3]

The aims of the present study were to assess whether the resting skin conductance level in babies was influenced by their state of arousal, and to study the effect of a 'heel prick', carried out for the purpose of blood sampling during clinical care, on the skin conductance level.

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Patients and methods

We studied 82 healthy babies whose gestational ages ranged from 25–42 weeks (median 32 weeks) and whose postnatal ages ranged from 1–73 days (median 8 days). Gestational age was assessed when possible from four sources: the date of the mother's last menstrual period, early antenatal ultrasound scan; clinical assessment after birth; and radiological bone age (babies on our unit who have a chest radiograph taken also routinely have a radiograph of one lower limb). Gestational age assessed by these methods did not vary by more than two weeks in individual babies, and therefore the mother's dates or early ultrasound assessment was used. We used the expression 'conceptional age' for gestational age plus postnatal age.

Cot nursed babies were lightly clothed under a double layer of blanket and the room air temperature ranged from 21°C to 25°C. Other babies were nursed in incubators at a constant ambient temperature. The rectal and skin temperatures of all babies were within the normal range at the time of study. No baby was visibly sweating at the time of the study and none was receiving anticholinergic drugs or other medications that might interfere with sweating. Measurements of the skin conductance level were timed to correspond with the need for blood sampling by heel prick.

Bipolar silver/silver chloride Beckman electrodes (diameter=4 mm) mounted in plastic cups were attached approximately 2·5 cm apart to the sole of a foot after cleaning the skin with distilled water and drying it. We used electrolyte medium with a 0·05 M sodium chloride concentration, which was freshly made every week, and we took care to avoid electrolyte seepage from under the electrodes. The electrodes were attached to a skin conductance meter which was constructed for us in the Department of Medical Physics, Central Manchester Health Authority. The equipment provided an alternating current of 10 Hz at a constant voltage of 100 mV, the maximum current permitted to flow between the two skin electrodes being 15 microamperes. Incorporated in the conductance meter, in series with the baby, was a resistor of negligible resistance (10 ohms) compared with the skin. The voltage measured across the series resistor was directly proportional to current flow which, in turn, was directly proportional to the skin conductance level. The meter incorporated a digital display of the skin conductance level within the range 0–20 µS (microsiemens), and permanent records were made by pen on a moving paper recorder. The conductance meter was calibrated before each recording session using resistors of known conductances.

After the skin electrodes were attached a 15 minute period of stabilisation was allowed before the 'preheel prick' level of skin conductance was read. We clinically assessed each baby's state of arousal when the reading was made, ascribing an arousal score as follows: 0=non-rapid eye movement sleep, no movements, regular breathing; 1=rapid eye movement sleep, small movements, irregular breathing; 2='quiet awake', eyes open, slight limb movements; 3='active awake', eyes open, moderate limb movements; and 4=crying and vigorous limb movements.

The heel prick was carried out on the contralateral foot to which the electrodes were attached and the calf was squeezed eight times to encourage blood flow to the heel. The procedure was carried out by the same person each time (GG). Recordings of the skin conductance level were continued for approximately 10 minutes after the heel prick. Our preliminary studies had shown that when a rise in the skin conductance level was observed after the heel prick the peak value almost always occurred at about one minute after the heel prick (fig 1). Therefore for the purpose of this study we measured the skin conductance level (from the permanent record) one minute after the heel prick and used this in the analysis of the results. The state of arousal was also documented one minute after the heel prick.

The Wilcoxon unpaired test was used to test the significance of comparisons between medians. Permission to study skin conductance in babies had been granted by the local ethics committee and parental consent was obtained in each case.

Results

Preheel prick skin conductance levels in babies less than 40 weeks' conceptional age had a relatively narrow range (0·1–0·7 µS) compared with the range in babies of 40 weeks or more among whom individual values were not normally distributed (fig 2). The median preheel prick skin conductance in babies within each conceptional age group less than 40 weeks (28–31 weeks; 32–35 weeks; 36–39 weeks) was identical (0·3 µS) and significantly lower compared with the level in babies 40–43 weeks (0·6 µS) (p<0·01). Within different conceptional age groups there was no significant correlation between the preheel prick skin conductance level and conceptional age, gestational age, or postnatal age.

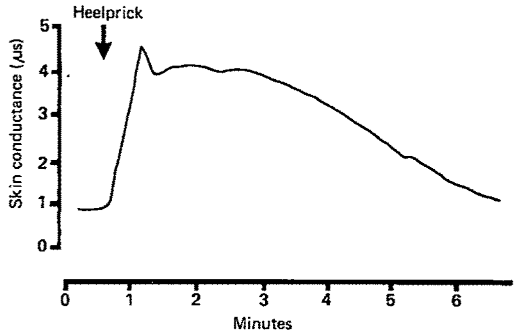

Figure 1 Skin conductance measured from the sole of the foot of a baby in response to a heel prick.

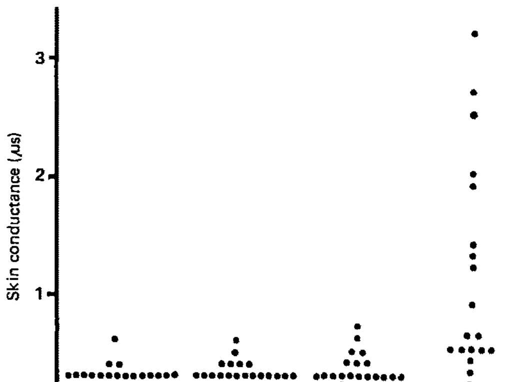

Figure 2 Preheel prick skin conductance levels in babies grouped according to their conceptional ages. Each point represents the result in an individual baby.

The distribution of the various states of arousal before stimulation by heel prick was similar in babies of different conceptional age groups (fig 3). Among babies 40–43 weeks' conceptional age, the preheel prick median skin conductance level was higher in those who were awake (states 2–4) compared with those who were asleep (0–1) (1·2 µS compared with 0·5 µS, p<0·03) (fig 4). In contrast, among babies less than 40 weeks' conceptional age the preheel prick median skin conductance level was identical in awake and asleep babies (0·3 µS).

All babies responded to the heel prick by becoming aroused. The proportion of babies who cried and showed vigorous activity (state 4) rose progressively from eight out of 17 (47%) in babies 28–31 weeks' conceptional age to 20 out of 22 (91%) in those of 40–43 weeks (fig 3). No baby had a fall in skin conductance level after the heel prick. In all babies less than 36 weeks' conceptional age the skin conductance level after the heel prick did not differ from the resting level. In contrast, in seven out of 23 (30%) babies 36–39 weeks' conceptional age, and in 21 out of 22 (95%) babies 40–43 weeks the skin conductance level rose after the heel prick. Among babies 36–39 weeks' conceptional age the proportion who cried vigorously after the heel prick was similar in those who had a rise in their skin conductance level (71%) and those who did not show a rise (69%). Among babies showing a rise in their skin conductance level the median (range) magnitude of the rise was significantly higher in babies 40–41 weeks' conceptional age (2·7 µS (0·2–6·3 µS)) compared with those 36–39 weeks (0·5 µS (0·1–1·4 µS), p=0·005, fig 5).

Among babies of conceptional ages 36–39 weeks and 40–43 weeks there was no correlation between the magnitude of the rise in skin conductance level and the resting skin conductance level, conceptional age, postnatal age, initial state of arousal, the state of arousal after heel prick, or the magnitude of the difference between the two states.

Discussion

Electrodermal activity may be measured as the conductance or the potential difference between two skin electrodes. In the former case it is necessary to pass a current through the skin from an external source (exosomatic) whereas in the latter no external current is required, the potential difference between an active and inactive skin electrode being generated from the skin itself.

We measured skin conductance using equipment that provided an alternating current at a constant voltage. When voltage is held constant the conductance of the circuit between the electrodes varies directly with the current through the skin. The advantages of using a constant voltage as opposed to constant current system, and of using an alternating current source rather than direct current have been reviewed by Venables and Christie.[4]

The mechanisms of electrodermal activity and the factors which influence it have been studied by many investigators.[4–7] In our study we measured skin conductance levels, but the physiological

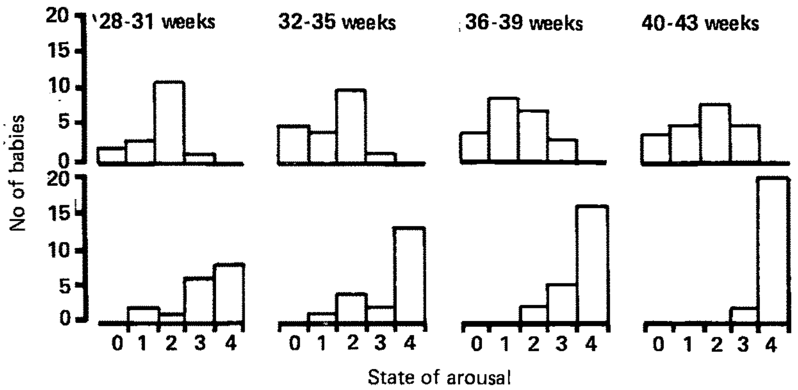

Figure 3 The number of babies, grouped according to conceptional ages, who showed different states of arousal before the heel prick (above) and after (below). State 0=non-rapid eye movement sleep, no movements regular breathing; 1=rapid eye movement sleep, small movements, irregular breathing; 2='quite awake', eyes open, slight limb movements; 3='active awake', eyes open, moderate limb movements; and 4=crying and vigorous limb movements.

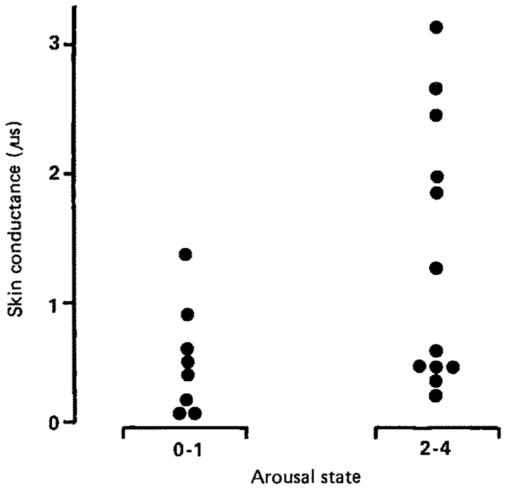

Figure 4 Preheel prick skin conductance levels in babies 40–43 weeks' conceptional age whose states of arousal were 0–1 compared with those 2–4. Each point represents the result in an individual baby. Comparison of median values: p=0·03.

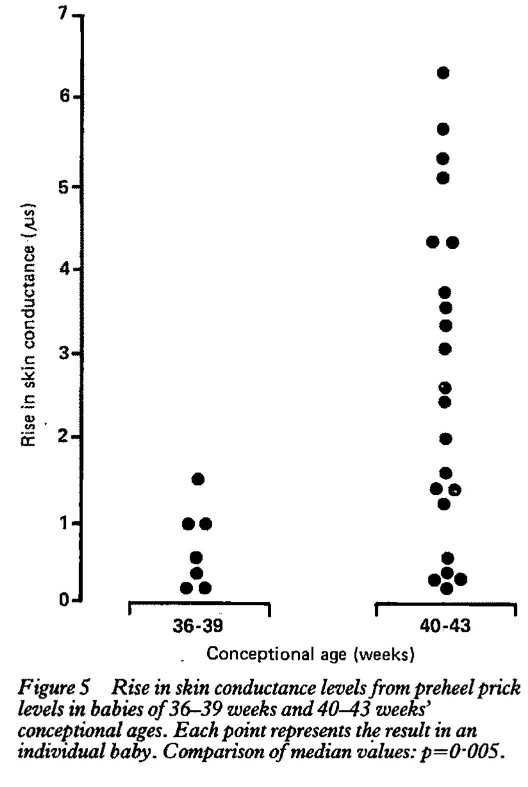

Figure 5 Rise in skin conductance levels from preheel prick levels in babies of 36–39 weeks and 40–43 weeks' conceptional ages. Each point represents the result in an individual baby. Comparison of median values: p=0·005.

principles are perhaps easier to understand by considering the concept of electrical resistance of the skin, the inverse of conductance. The high resistance to the passage of an electrical current across the skin is a function of the properties of the epidermis. When the sweat ducts become filled, the resistance of the skin is lowered and in this respect each sweat duct behaves like a resistor in parallel. In addition, filling of the ducts is associated with hydration of the stratum corneum, due in part to reabsorption of water from the sweat ducts, and this also contributes to the lowering of skin resistance, the hydrated stratum corneum behaving like a resistor in parallel with the sweat ducts. Skin resistance is also influenced by structures that behave as resistors in series, and these include dry stratum corneum and all tissues beneath it excluding the lumen of the sweat glands and ducts.[8]

We have previously shown that in term babies who are quietly awake evaporative water loss measured from the sole of a foot correlated positively with the skin conductance level measured simultaneously from the other foot[9]. Others have shown that cholinergic blocking drugs cause a fall in skin conductance levels.[10]

The low level of skin conductance we observed in babies less 36 weeks' conceptional age presumably reflects electrical conduction largely through a dry stratum corneum and other epidermal tissues, including the walls but not lumens of the sweat ducts. Muramatsu *et al* measured skin resistance from the abdominal wall and showed that it was very low (that is, high conductance) at 25 weeks' gestation and rose exponentially (that is, conductance fell) with advancing gestational age.[11] At first sight these results appear to be at variance with our own but this difference is readily explained. Their measurements largely reflect resistance of the epidermis, especially the stratum corneum, which is very thin in preterm babies and thickens as it matures, resulting in an increase in its electrical resistance. The authors excluded all babies who were visibly sweating and indeed one would not have expected their very preterm babies to have significant sweat gland activity in their abdominal wall skin. As the authors state the added factor of sweat gland activity (which would reduce resistance) was probably negligible. In contrast, our measurements of skin conductance were made from the palm and soles which even in preterm babies are relatively thick. Skin conductance at these sights largely reflects the activity of the sweat glands.

Even when observed to be aroused by a painful stimulus none of our babies less than 36 weeks' conceptional age had a rise in the skin conductance level suggesting that arousal mechanisms and pathways (including peripheral mechanisms) which promote emotional sweating were still immature. Certainly by 28 weeks' gestation babies have a full complement of sweat glands. Although it is well known that the sweat glands are innervated by the sympathetic nervous system, the precise central influences on emotional sweating and skin conductance are not known. Psychophysiologists speak of a 'hierarchy of suprasegmental control' including not only the cerebral cortex but the limbic system, the reticular activating system, and the spinal cord,[4] but much of the experimental work has been in the context of phasic skin conductance responses rather than tonic levels of skin conductance.

No baby less than 36 weeks' conceptional age had a rise in their skin conductance levels after being aroused by a painful stimulus, whereas all except one of the 22 babies of 40–43 weeks, and seven out of 23 babies of 36–39 weeks showed a rise. While it is true that a smaller proportion of babies less than 36 weeks were observed to cry vigorously after the heel prick this cannot account for failure of their skin conductance to rise because no rise was observed even among those babies who did cry vigorously. Furthermore among babies 36–39 weeks' conceptional age the proportion who cried vigorously after the heel prick was similar in those who had a rise in their skin conductance level (71%) and those who did not show a rise (69%).

Our results are in keeping with the notion that the skin conductance level rises after an arousal stimulus only in these babies who are mature enough to have developed the capability of emotional sweating and that this first occurs around 36–39 weeks' conceptional age. In support of this, only in babies 40–43 weeks' conceptional age did we observe an association between their state of arousal (awake or asleep) before stimulation and their level of skin conductance. It seems that mature babies can modulate their skin conductance level in accordance with their state of arousal whereas immature babies have a relatively constant skin conductance level regardless of their state.

These observations are consistent with those of Harpin and Rutter who showed that emotional sweating from the palm, measured as evaporative water loss in response to a heel prick, did not develop until 36 weeks after conception.[12] In the same study these authors observed a positive relationship between palmar water loss and level of arousal which first appeared in infants of 36–37 weeks' conceptional age. In a previous study in which we recorded skin conductance levels for several hours in individual babies we showed that among babies of 37 weeks' conceptional age or more the mean skin conductance level during awake periods was higher than in sleep periods, whereas a significant difference was not seen among less mature babies.[9]

In the present study, among those babies who had a rise in their skin conductance level after arousal by a painful stimulus the magnitude of the rise was significantly higher in babies of 40–43 weeks' conceptional age than babies 36–39 weeks. This is an important observation suggesting that the onset of emotional sweating is not an all or none phenomenon. It would seem that the less mature babies, after arousal by heel prick, had a fewer number of actively excreting sweat glands, or the volume of sweat per duct was smaller, or hydration of the stratum corneum was less. In terms of the contribution to skin conductance levels it is difficult to distinguish between activity of the sweat glands and hydration of the stratum corneum as the former leads to the latter.

Our study does not address the response of the newborn baby specifically to pain, although pain was used (opportunistically) to stimulate arousal. Crying and other somatic disturbances indicating arousal occur in response to numerous psychic stimuli as well as to pain. Conversely adults certainly have the ability to suppress somatic manifestations of pain while nonetheless still feeling it. We observed that although immature babies become aroused and may cry in response to a heel prick, they show no rise in their skin conductance level presumably because they are incapable of emotional sweating.

We think it most unlikely that we failed to observe a late rise in skin conductance levels among these babies. Although in this study the skin conductance level was recorded at one minute after the heel prick we continued to observe the level for 10 minutes.

It would appear at first sight that the study of electrodermal activity might have only limited application to the study of pain in immature babies. Although we measured the change of skin conductance levels in response to a painful stimulus we did not measure the 'skin conductance response', which is a different entity used by psychophysiologists to describe a phasic change in skin conductance occurring with a latency of 1 to 2·5 seconds after stimulation, with a time to peak response of less than one second, and an amplitude of 0·01–5·0 μS. It was not our aim to study skin conductance responses and our equipment was not designed for this purpose. We cannot rule out the possibility of there being rapid phasic electrodermal activity in response to emotional arousal which has so far gone unnoticed in immature babies and we are exploring this further.

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Gut blood flow velocities in the newborn: effects of patent ductus arteriosus and parenteral indomethacin

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Abstract

The effects on gut blood flow velocities of parenteral indomethacin (0·2 mg/kg) given either quickly as a bolus or slowly as an infusion were compared in consecutive studies of two groups of infants with symptomatic patent ductus arteriosus. In the presence of patent ductus arteriosus the range of velocities in the superior mesenteric artery before indomethacin was given was characterised by pronounced abnormalities including absent—or in some cases even retrograde—diastolic flow.

In eight subjects the first rapidly given bolus dose of indomethacin (duration 20 seconds or less) caused a pronounced and sustained fall in the velocity of the superior mesenteric artery blood flow (mean peak systolic velocity (cm/second): before 74; after 38; median time to maximum fall 7·4 minutes; median time to recovery 50 minutes). A further 10 subjects received their first dose of indomethacin by slow infusion (duration 30–35 min) and the percentage fall in peak systolic velocity was both substantially less (22% compared with 47%) and later (median time to maximum fall 37·3 minutes) than after rapid infusion. Qualitatively similar but smaller changes were seen in the coeliac axis. Return of antegrade end diastolic flow in the superior mesenteric artery within one hour of the first dose of indomethacin was a good predictor of subsequent closure of the ductus.

These data suggest that there is a profound disturbance in mid gut perfusion in infants with patent ductus, which is exacerbated by indomethacin given rapidly by intravenous bolus. They may also provide a rational explanation for the well recognised association between necrotising enterocolitis and both patent ductus arteriosus and indomethacin administration. The unwanted effects of the indomethacin are abrogated by slow infusion, without loss of efficacy in closure of the ductus.

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The role of indomethacin in the management of patent ductus arteriosus in preterm neonates is well established.[1 2] Its use is associated with unwanted gastrointestinal effects, however, including intestinal haemorrhage, local perforation, and necrotising enterocolitis. The reason for these is not known, and they have been described in association with oral, rectal, intramuscular, or intravenous administration.[3–7] In addition local ileal perforation in neonates has been described after indomethacin has been used to inhibit preterm labour in their mothers.[8]

Indomethacin has well recognised effects on the cerebral circulation. Intravenous boluses reduce cerebral blood flow in the adult human,[9] and cerebral blood flow velocity in the newborn.[10 11] No deterioration in cerebral function has yet been reported in association with bolus indomethacin, however, although the fall in blood flow velocity has been considered undesirable.

To date there is no direct evidence that intestinal ischaemia plays a part in the pathogenesis of necrotising enterocolitis in man, but Doppler studies in animals[12 13] and on severely growth retarded human fetuses suggest that this is a possibilty.[14] It is therefore possible to hypothesise that the gastrointestinal side effects of indomethacin may be mediated by a disturbance in gut perfusion.

We have previously used Doppler ultrasound to study gut blood flow velocities in human infants born at full term and have described the changes in splanchnic circulation with increasing postnatal age and in response to feeds. (RC Coombs, MEI Morgan, GM Durbin, *et al*; unpublished observations).[15]

Before this study it was our practice to give indomethacin as an intravenous bolus. The present studies were designed to determine the effect of a rapidly administered bolus of indomethacin on the splanchnic circulation, and whether such changes could be reduced by slow infusion in infants with symptomatic patent ductus arteriosus.

Subjects and methods

CONTROL SUBJECTS

In a preliminary study, only those subjects with patent ductus arteriosus were shown to have absent end diastolic forward flow. Control subjects covering a wide range of birth weights and gestational ages were therefore chosen to find out whether absent end diastolic forward flow was present in those who did not have clinically apparent patent ductus arteriosus.

Eighteen preterm infants without symptomatic patent ductus arteriosus were studied (table 1). They were not matched with the study subjects for maturity, weight, or postnatal age. They were significantly more mature ($p<0·003$), heavier ($p<0·002$), and studied earlier than the study subjects. Each was studied on several occasions (range 1–6) during the first week of life.

STUDY SUBJECTS

Subjects were entered into the study if a clinical

Table 1 Clinical details of control subjects and those with symptomatic patent ductus arteriosus receiving either an intravenous bolus dose or a slow infusion of indomethacin

	No of control subjects (n=18)	*No who received bolus dose (n=9)*	*No who received slow infusion (n=10)*
Mean (range) gestational age (weeks)	31·2 (27–35)	27·3 (23–31)	28·7 (24–35)
Mean (range) birth weight (g)	1460 (950–2890)	1090 (720–1990)	1190 (630–2670)
Mean (range) age at time of study (days)	2·7 (1–6)	12·7* (6–21)	8·5* (3–12)

*p<0·02.

decision was made to give indomethacin (in three doses 12 hours apart) for the treatment of symptomatic patent ductus arteriosus.

STUDY 1: INDOMETHACIN BY BOLUS

Nine preterm neonates (table 1) were studied on 10 occasions (one infant received a second course of indomethacin). One infant was later found to have tetralogy of Fallot, and data from this patient have been analysed separately.

STUDY 2: INDOMETHACIN BY SLOW INFUSION

Ten preterm infants were also studied who received indomethacin by slow intravenous infusion over 30–35 minutes (table 1). There were no significant differences between these subjects and the first group in gestation or birth weight, although they were studied earlier than the first group (mean 4 days).

DOPPLER ULTRASOUND

A Hewlett Packard duplex pulsed Doppler ultrasound system with a 5 MHz short focus probe and high pass filter at 50 Hz was used to visualise the coeliac axis and superior mesenteric artery. Blood flow velocities were measured from a point just distal to the origin of these vessels at the aorta. The range of velocities from each vessel were recorded on videotape for later analysis by the analysis package contained within the Hewlett Packard system. The duplex system allowed measurement of the angle of insonation, which was kept between 0 and 40°. For analysis of the ranges at any time point, the following were measured over six consecutive cardiac cycles: (i) peak systolic velocity; (ii) end diastolic velocity; (iii) time averaged mean velocity; and (iv) the area under the peak velocity envelope. In another study we showed that the measurement with the least variation is the peak systolic velocity (RC Coombs, MEI Morgan, GM Durbin, *et al*; unpublished observations).

We were unable to determine accurately the diameter of the superior mesenteric artery or coeliac axis, and cannot therefore comment on changes in volume flow.

CLINICAL MANAGEMENT

Clinical management of the subjects with patent ductus arteriosus included fluid restriction to 90 ml/kg for 24 hours before the indomethacin was given. Any enteral nutrition was stopped for the hour before the indomethacin was given, and was not restarted until after the study.

ADMINISTRATION OF INDOMETHACIN

Indomethacin trihydrate (Indocid PDA; Thomas Morson) was given in intravenous doses of 0·2 mg/kg on three occasions, with 12 hour intervals between doses. Only velocity data concerning the first dose were used in the analysis.

Indomethacin by bolus

For rapid infusion the indomethacin was diluted to 1 mg/ml with water or 5% dextrose and given as a bolus in 20 seconds or less.

Slow infusion of indomethacin

For slow infusion, the indomethacin was diluted with water or 5% dextrose to a concentration of 0·2 mg/ml and infused over 30–35 minutes with a constant infusion pump.

In each study, baseline velocity measurements were recorded from the coeliac axis and superior mesenteric artery in the fasting state before, and for a period up to two hours after, the indomethacin was given.

After both the bolus dose and the slow infusion of indomethacin we calculated the time taken to reach the maximal change in velocity, and the magnitude of that change.

STATISTICAL ANALYSIS

The significance of differences between the groups was assessed with the Mann-Whitney U test, Student's paired *t* test, or Fisher's exact test.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Research Ethics Committee of the Central Birmingham Health Authority. Informed consent was obtained from parents before each study.

Results

VELOCITY PROFILES BEFORE INDOMETHACIN WAS GIVEN

Superior mesenteric artery

Controls: none of the control subjects had a clinically suspected patent ductus arteriosus. Three had absent end diastolic forward flow on day 1. Subsequently all control subjects had velocity ranges with forward flow throughout the entire cardiac cycle (fig 1A).

Study subjects: In contrast to the controls, the

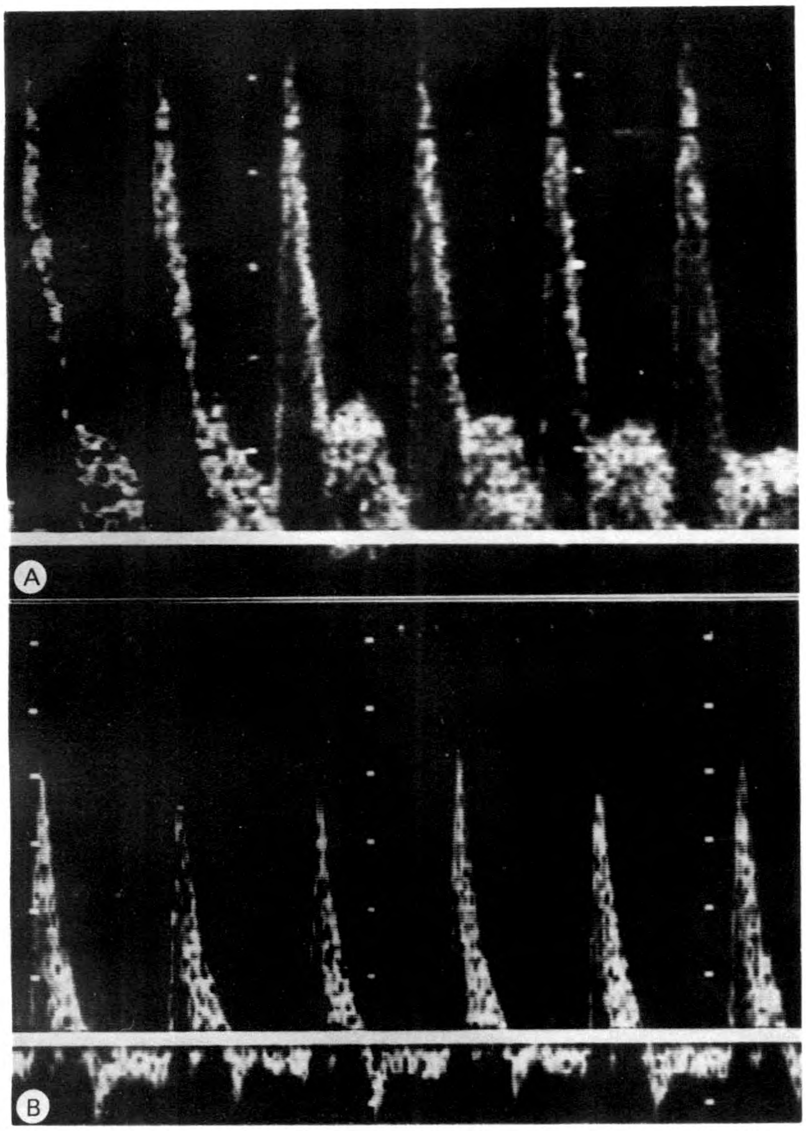

Figure 1 Typical ranges of superior mesenteric artery velocity (A) in a control child, and (B) in an infant with a symptomatic patent ductus arteriosus. Ranges above the line indicate antegrade, and below the line retrograde, flow. Note the retrograde diastolic flow in (B).

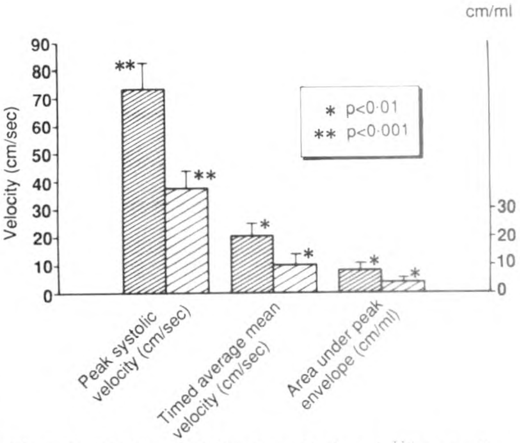

Figure 2 Effects of a bolus dose of indomethacin on peak systolic velocity, time averaged mean velocity, and area under peak velocity envelope in nine infants. Close hatched bars indicate minimum values in the superior mesenteric artery before indomethacin was given, and wide hatched bars indicate the values after administration of indomethacin. Values are mean (SD).

velocity ranges in subjects with symptomatic patent ductus arteriosus were characterised by absent end diastolic forward flow, or in some cases retrograde diastolic flow (flow out of the superior mesenteric artery during diastole) (fig 1B).

Coeliac axis

Controls: At no time during the study periods was there absent or retrograde diastolic flow in any of the control subjects.

Study subjects: Retrograde flow was rarely seen in the coeliac axis, and in only eight cases (four in each study group), was there absent end diastolic forward flow.

EFFECTS OF INDOMETHACIN

Indomethacin by bolus

Indomethacin led to a rapid and profound fall in peak systolic velocity, time averaged mean velocity, and area under the peak velocity envelope in the superior mesenteric artery (fig 2), such that the mean (SD) peak systolic velocity fell from 74 (30) cm/sec before indomethacin was given, to 38 (13) cm/sec after the bolus ($p<0·008$).

In the coeliac axis the changes in peak systolic velocity were qualitatively similar but the magnitude of the fall was substantially less, being 63 (19) cm/sec before indomethacin was given and 43 (16) cm/sec after the bolus ($p<0·03$). The median time to the maximal fall in peak systolic velocity was 7·4 minutes (range 1·4–16) in the superior mesenteric artery and 8·0 minutes (range 3·5–26) in the coeliac axis.

After this profound fall in velocity within the superior mesenteric artery, the median time to recovery was 50 minutes (range 15–191). In the five subjects whose recovery times were longer than 50 minutes closure of the ductus was successful. In contrast, indomethacin did not permanently close the ductus in three of the four subjects whose recovery time for peak systolic velocity was less than 50 minutes.

Slow infusion of indomethacin

After slow infusion of indomethacin, the fall in peak systolic velocity in the superior mesenteric artery was not significant, falling from a mean (SD) of 71 (24) cm/sec to 56 (32) cm/sec at the maximum fall in velocity. Similarly there was no significant change in coeliac artery peak systolic velocity, being 63 (19) cm/sec before indomethacin was given, and 66 (33) cm/sec at the inflection of the velocities.

As the range of velocities before indomethacin was given in both studies was wide we have compared the percentage fall instead of the actual fall in peak systolic velocity between the two studies. The percentage fall of peak systolic velocity in the superior mesenteric artery after the bolus dose of indomethacin was significantly greater than the fall associated with the slow infusion of indomethacin: 47% (range 20 to 63) with the bolus of indomethacin compared with 22% (range −33 to +53) with the slow infusion ($p<0·02$). The fall in peak systolic velocity was

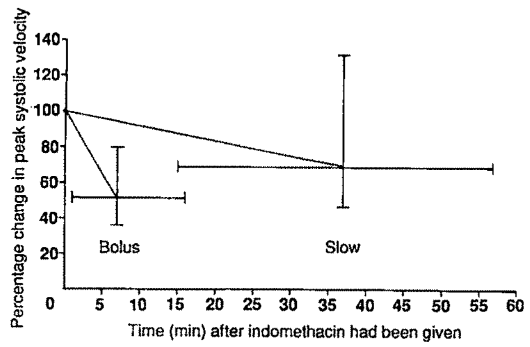

Figure 3 Comparison of magnitude and time course of changes in peak systolic velocity in the superior mesenteric artery after bolus and slowly infused doses of indomethacin.

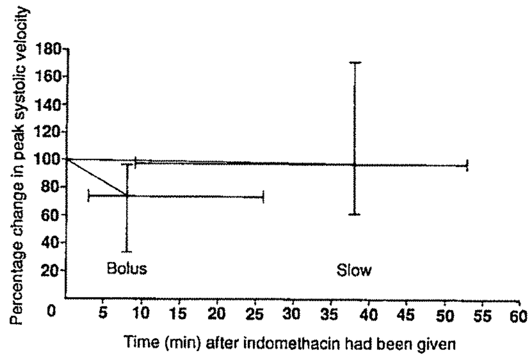

Figure 4 Comparison of magnitude and time course of changes in peak systolic velocity in the coeliac axis after bolus and slowly infused doses of indomethacin.

also greater in the coeliac axis after the bolus dose of indomethacin: (31% (range 3 to 65) after the bolus dose of indomethacin compared with −3% (range −72 to +39) with the slow infusion ($p<0\cdot04$)).

After the slow infusion of indomethacin the time to the maximal change in velocity was also significantly longer in both the superior mesenteric artery and coeliac axis. In the superior mesenteric artery it was 37 minutes after the slow infusion, compared with 7·4 min after the bolus ($p<0\cdot003$). At the coeliac axis it was 38 minutes after the slow infusion, compared with 8 minutes after the bolus ($p<0\cdot0013$) (figs 3 and 4).

Return of antegrade diastolic flow

Return of antegrade diastolic flow after the first dose of indomethacin was the first clinical indication of the likelihood of closure of the ductus. Among the 18 subjects studied on 19 occasions there was a close association between return of antegrade diastolic flow within one hour of the first dose of indomethacin and subsequent clinical ductal closure ($p<0\cdot002$; table 2).

Table 2 Association between return of antegrade end diastolic flow in the superior mesenteric artery within one hour of the first dose of indomethacin (bolus or slowly infused) and subsequent ductal closure after 19 courses of indomethacin ($p<0\cdot002$)

	Ductus closed	*Ductus reopened or no change*
Return of end diastolic volume within one hour	11	1
No return of end diastolic volume within one hour	2	5

Efficacy and side effects

There was no apparent loss of efficacy with the slow infusion of indomethacin. Seven of 10 ducts closed clinically, one reopened and was ligated, and two remained open. Of the eight subjects who received bolus doses of indomethacin, closure of the ductus occurred in five with the first course, in one with the second, and two remained open. No serious gastrointestinal side effects were seen; one subject in the bolus group developed a transient paralytic ileus.

Discussion

Work in experimental animals together with the known clinical risk factors indicate that ischaemia probably plays at least a part in the pathogenesis of necrotising enterocolitis. In newborn piglets, for example, significant falls in blood flow were seen after severe acute asphyxia,[12] as well as after a less severe prolonged period of asphyxia.[13] On histological examination in each case there was evidence of ischaemia with congestion, haemorrhage,[12] and areas of necrosis.[13]

A patent ductus is a known risk factor for necrotising enterocolitis.[15] Our observations on gut blood flow velocities in preterm infants lead us to believe that the absent or retrograde diastolic flow found in infants with symptomatic patent ductus arteriosus represents a compromised splanchnic circulation, and suggests a possible mechanism whereby a patent ductus may predispose to necrotising enterocolitis.

Absent forward end diastolic flow in the superior mesenteric artery seems to indicate patent ductus arteriosus. It was seen in all subjects with a symptomatic patent ductus arteriosus, and in only three of the control subjects on day 1 only—a time when an asymptomatic patent ductus commonly occurs.

Indomethacin by bolus leads to a profound and sustained fall in splanchnic blood flow velocity, further compromising the vascular bed. The mechanism of the fall in velocity is not known, although similar changes have also been seen in the splanchnic circulation of cats and dogs after they were given indomethacin.[16 17] In cats the response was dose related and not inhibited by previous exposure to the known cycloxygenase inhibitor, meclofenamic acid. In dogs profound mesenteric vasoconstriction was seen after indomethacin had been given, but not after ibuprofen, which has a similar inhibitory effect on cycloxygenase. This supports the view that the vasoconstricting effect of indomethacin on the splanchnic circulation is independent of its actions on prostaglandin metabolism.

In two infants in whom the effects of all three bolus doses of indomethacin were recorded, we noted that the vasoconstricting response became progressively less. The percentage maximum fall in velocity decreasing from 58% to 7% and 50% to 16%. Similar effects have been reported in the cerebral circulation of adult man,[9] but have not been examined in the cerebral circulation of neonates.

Necrotising enterocolitis, local intestinal perforation, and haemorrhage have all been documented in neonates receiving both parenteral and enteral indomethacin. Indomethacin given rectally in dogs profoundly reduced mucosal blood flow to the stomach, and the mid and distal ileum, suggesting that changes in blood flow may be just as important after indomethacin has been given enterally.[18]

In the infant with tetralogy of Fallot in our study there was good antegrade diastolic flow before indomethacin had been given (7·2 cm/sec). After the indomethacin had been given there was a pronounced fall in peak systolic velocity of 58%, which recovered after one hour. There was no apparent change in the clinical condition. This provides further evidence that indomethacin has effects on the splanchnic vascular bed which are independent of its actions on the ductus.

Our data suggest that the vasoconstricting effects of a bolus dose of indomethacin on a splanchnic vascular bed that is already compromised by a patent ductus arteriosus provide at least one explanation for the gastrointestinal side effects of indomethacin. This effect of a bolus dose of indomethacin can be avoided by giving the drug by slow infusion. No adverse gastrointestinal effects were seen when indomethacin was infused slowly, nor was there any apparent loss of efficacy in closure of the ductus. One infant with abdominal distension and blood and mucus in his stool, but with no intramural gas on abdominal radiograph, was successfully managed with a slow infusion of indomethacin.

We thank the medical and nursing staff of the neonatal unit at the Birmingham Maternity Hospital, and Melvyn Docker and Phil Williams of the department of medical physics for technical help.

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Hiccups and breathing in human fetuses

Mary Pillai, David James

Abstract

Serial recording in 45 low risk fetuses throughout the second and third trimesters showed that hiccups were the predominant diaphragmatic movement before 26 weeks' gestational age and that there was a significant negative correlation with gestational age. There was a pronounced reduction between 24 and 26 weeks, which was the result of a decrease in the number of episodes of hiccups rather than a change in the duration of episodes. In contrast, fetal breathing was positively correlated with gestational age, the greatest increase in breathing occurring between 26 and 32 weeks' gestation. This was the result of both an increase in the number and duration of episodes. From the time that rest-activity cycles of behaviour could be determined in recordings, both breathing and hiccups were dependent on behavioural state or cycle, occurring predominantly during active episodes. This association between quiet and active behaviour and breathing did not alter with increasing gestational age, and the variables in fetal behavioural state became increasingly closely linked.

The importance of prolonged and repeated recording, and also the need to take account of other variables in fetal behaviour, before any sinister conclusions can be drawn about the absence of fetal breathing is emphasised.

The human fetus makes a number of different diaphragmatic movements. With the notable exception of fetal breathing, however, there are virtually no data on these. Other types of diaphragmatic movement that have been described include sighs, hiccups, and gasping. Of these fetal hiccups are the most notable, but in contrast to breathing they have received scant attention and knowledge of the physiological mechanisms underlying their control and the part they play in fetal development is lacking.

Since the recognition that intermittent breathing activity is a normal feature of fetal life with a tendency to be stimulated by carbon dioxide and inhibited by hypoxia,[1 2] many data have been published on fetal breathing in experimental animals using invasive techniques, especially in fetal lambs. With the development of non-invasive methods of fetal observation, interest in fetal breathing extended to it being used as an indicator of wellbeing in human fetuses.[3 4] Suppression of fetal breathing has been suggested to be an early predictor of fetal infection in patients with ruptured membranes,[5] as a predictor of true preterm labour in pregnancies complicated by preterm contractions,[6] and as a predictor of the development of pulmonary hypoplasia in fetuses at high risk.[7 8]

All this has occurred despite little published longitudinal data about human fetuses, particularly in normally developing low risk fetuses, and so it is not surprising that those few studies in which fetal breathing in pathological circumstances has been matched with that in normal control fetuses,[9 10] the results conflict with observations of fetal breathing in pathological circumstances only.[7]

The aims of this study were to document longitudinally, in normally developing low risk human fetuses, developmental trends in fetal breathing and hiccups, their association with developing patterns of fetal behaviour, and the range of individual variations.

Patients and methods

Women with no history of hypertension, renal disease, or diabetes and who had previously delivered at least one healthy normally grown infant at full term, and women having their first babies who had similar medical histories were invited to participate in a longitudinal study of multiple fetal variables. All those recruited smoked less than 10 cigarettes/day and each had a viable singleton pregnancy confirmed by ultrasound during the first 16 weeks of pregnancy.

Twenty one primigravidas and 24 multigravidas were recruited. Recordings were started between 14 and 18 weeks' gestation and repeated at monthly intervals until delivery. Fetal diaphragmatic movements and limb, body, and eye movements were detected with real time ultrasound and recorded polygraphically at a paper speed of 3 cm/minute. The fetal heart rate was also recorded ultrasonically using a standard fetal heart rate monitor (Hewlett Packard 8040A) with an output to the polygraph to allow synchronising of all variables. During most of the second trimester, recordings were made with a single scanner (Siemens Sonoline SL1 with a 3·5 MHz transducer), but in the last trimester two scanners were used, one giving a parasagittal view of the fetus's face (Siemens Sonoline SL1 with a 3·5 MHz transducer) and the other giving a transverse view of the fetus's abdomen (Toshiba SAL 20A with a 2·4 MHz transducer). Fetal eye, somatic, and mouthing movements, and breathing and hiccups, were recorded on separate channels of the polygraph (Hewlett Packard 7754A) with mulitple hand held push buttons.

All recordings were made by a single observer

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(MP) in a quiet darkened room with the mother seated in a comfortable semirecumbent position, for which we found a Parker Knoll chair most suitable.

Recordings were undertaken between 0900 and 1800, and continued for 90–100 minutes. Within individual subjects recordings were repeated at the same time of day and at the same interval after meals, and the four women who smoked did not do so during, or for two hours before, recording. All participants were outpatients with no risk factors known to affect fetal behaviour or breathing.

The following definitions were used:

Fetal breathing: contraction of the diaphragm resulting in downward and outward displacement of the abdominal contents and inward displacement of the thorax. All episodes of fetal breathing were counted providing they comprised a minimum of four consecutive breaths sustained for at least five seconds. All episodes in which the breath to breath interval lasted for six seconds or more were excluded.[11] Isolated diaphragmatic movements were not counted.

Fetal hiccups: characterised by more forceful and regular diaphragmatic contractions at a slower rate than fetal breathing. All episodes of hiccups comprising a minimum of five hiccups in 30 seconds, and sustained for 30 seconds, were counted.

Rest-activity cycles: a relatively high base rate of fetal movements interrupted by a period or periods of quiescence lasting six minutes or more (see below).

Behavioural states: stable association of heart rate pattern and the condition of eye movements and somatic movements for intervals of at least three minutes duration with simultaneous transition (within three minutes) of all three variables at times of transition between states.[12]

Statistical analysis of the data was by analysis of variance and the paired Student's t test. Approval for the study was obtained from the hospital's research and ethics committee.

Results

A total of 268 recordings was made (mean 6·0, range 4–7/fetus) and the total recording time was 402·4 hours.

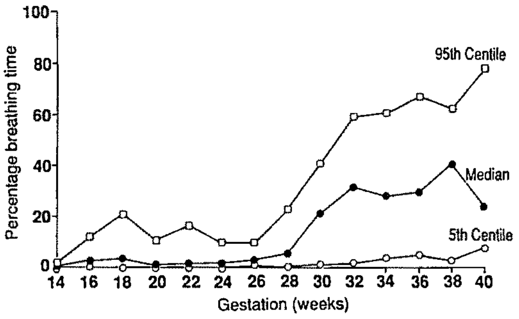

Figure 1 Median and centile values for the percentage of time fetal breathing was observed during 100 minute observation intervals every four weeks between 14 weeks and full term in 45 healthy fetuses.

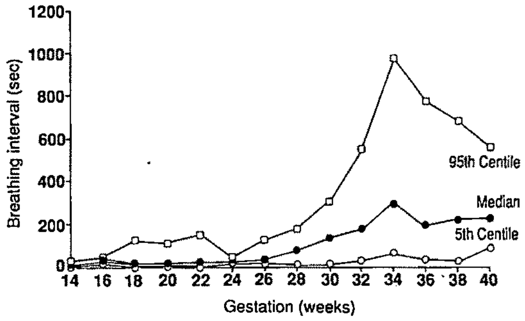

Figure 2 Median and centile values for the longest sustained intervals of fetal breathing (secs) in 45 healthy fetuses between 14 weeks and full term.

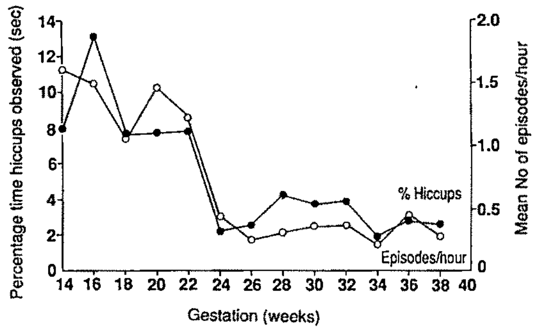

Figure 3 Mean percentage of time during which hiccups were observed ($r=-0{\cdot}33$), and the mean number of episodes of hiccups/hour ($r=-0{\cdot}47$) in 45 healthy fetuses between 14 weeks and full term.

BREATHING

Before 26 weeks' gestation 105 recordings were made and these were characterised by a low percentage of fetal breathing movements (median 1·4%, range 0–26%), with no breathing at all observed during 45 (43%) of the recordings. Between 26 and 30 weeks' gestation there was a pronounced increase in the percentage of breathing with a continuing upward trend until 36 weeks' gestation (fig 1). The longest sustained intervals of fetal breathing showed a similar upward trend, but peaked 2–4 weeks earlier (fig 2).

HICCUPS

Fetal hiccups showed a different developmental course, being significantly more common before 24 weeks' gestation than subsequently ($p<0{\cdot}0001$), and this was solely because there were more episodes of hiccups before 24 weeks' gestation (fig 3). We did not find any developmental trends in the duration of episodes of hiccups after 18 weeks' gestation (fig 4). There was no apparent association between patterns of hiccups and breathing.

INDIVIDUAL FETAL VARIATION

With the exception of the period 32–36 weeks' gestation there were individual fetuses in which no breathing at all was observed during recording at all gestational ages. Similarly, with the exception of 33–34 weeks' gestation there were individual fetuses in which no intervals of breathing were sustained for 30 seconds, and at all

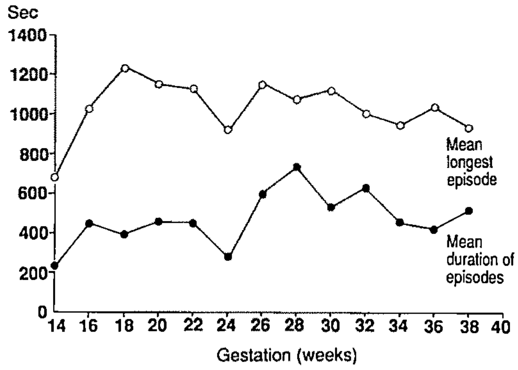

Figure 4 Mean duration of episodes of hiccups and mean duration of longest observed episode between 14 and 40 weeks gestation.

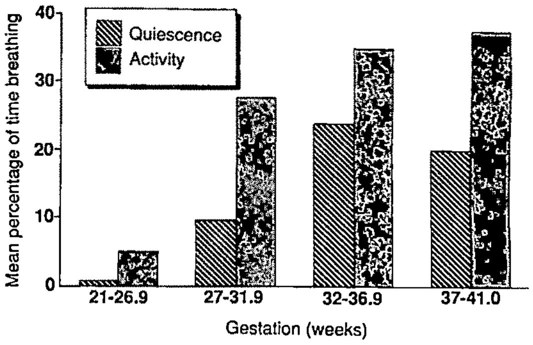

Figure 6 Mean percentage of time breathing was observed during rest and activity during only those recordings containing rest activity cycles (137 of 267 recordings). The differences between rest and activity were significant ($p<0\cdot001$, paired t test).

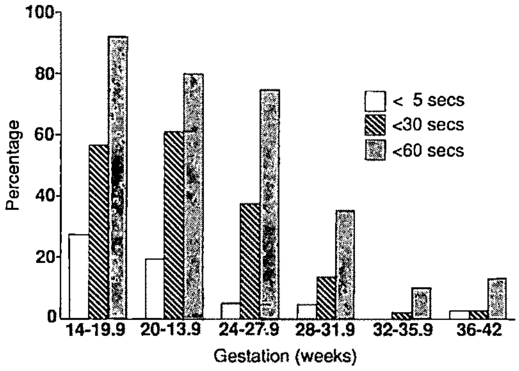

Figure 5 Percentage of recordings of <5 secs, <30 secs, and <60 secs of sustained fetal breathing in 260 recordings on 45 fetuses, with each fetus represented no more than once in each 4 week age group.

gestational ages there were fetuses in which no intervals were sustained for 60 seconds (fig 5). Breathing was observed during at least two recordings of each individual fetus. There was also considerable individual variation in hiccups, with several fetuses having no episodes in any of their recordings, and one had one episode or more during every recording.

ASSOCIATION WITH BEHAVIOURAL STATES AND CYCLES

With advancing gestational age there was a significant increase in the longest intervals of complete quiescence ($p<0\cdot001$), so that alternating cycles of rest and activity became more apparent. Using the arbitrary definition of six minutes for the minimum duration of a rest cycle, rest-activity cycles were rarely observed before 24 weeks' gestation, but by 29 weeks they were present in over 80% of recordings. Fixed and recurring association of body movements, eye movements, and fetal heart rate patterns into well defined fetal behavioural states were first observed in one fetus at 33 weeks' gestation and were present in over 80% of recordings by 36 weeks.[12]

Throughout the range of gestation studied, fetal breathing movements were significantly more common in the active cycles ($p<0\cdot001$) (fig 6), but within individual fetuses this finding was not consistent, with six of the 137 recordings that contained rest and activity cycles (4%) containing more breathing during quiescence than during the active cycles.

The onset of fetal hiccups was only observed during active cycles. In two recordings hiccups continued for several minutes in quiescence when a transition between cycles occurred shortly after their onset, but otherwise hiccups did not occur during periods of quiescence.

FETAL OUTCOME

All mothers delivered between 37 and 42 weeks' gestation. All one minute Apgar scores were 5 or more, and five minutes scores were 7 or more. The mean (SD) mean birth weight was 3532 (435) g (range 2700–4650). One infant was admitted to the neonatal unit for investigation of pyrexia that settled without treatment, and one other infant died suddenly and inexplicably at 8 weeks. Further careful retrospective examination of the recordings of this infant did not uncover any differences compared with the group as a whole. All other infants were between 9 months and 2 years of age at the time of writing, and developing normally.

Discussion

The onset of both hiccups and breathing have been described during the first trimester, roughly one to two weeks after the development of the diaphragm[13]; their development thereafter, however, differs considerably, fetal hiccups being the predominant type of diaphragmatic movement until 26 weeks' gestation, after which fetal breathing predominates. This suggests that the centres that control fetal breathing are not only different, but also probably more complex, than those that control hiccups.

The early appearance of both types of activity suggests their ontogeny may depend only on a simple reflex, but the subsequent developmental course of breathing and experimental destructive lesions in fetal lambs shows that the development of higher centres has a profound influence on fetal breathing.[14, 15] Such data about the developmental trends and influence of higher centres on hiccups is lacking, but our observations suggest that if subsequent development of the brain does influence hiccups the effect may well be inhibitory. Anecdotal evidence that supports this is our observation of hiccups continuing in compromised fetuses long after other types of normal behaviour, including breathing, have ceased (unpublished observations).

Van Woerden *et al* analysed hiccups in a cross sectional study of healthy fetuses born at full term.[16] The frequency of episodes and percentage of recording time for hiccups were the same as we observed at comparable periods of gestation, but in contrast to our findings they found no specific association with behavioural states.

The data of Wigglesworth and Desai provided convincing evidence that breathing movements play an important part in the development of fetal lungs.[17 18] Patrick *et al* have previously documented fetal breathing in the last 10 weeks of pregnancy, but their data are from single rather than serial studies.[11 19] They drew attention to the fact that prolonged episodes of apnoea are characteristic of healthy human fetuses. Our data show that this is particularly so before 30 weeks' gestation when—during prolonged recording—more than 40% of healthy fetuses have no breathing, and about 60% do not sustain breathing continuously for 30 seconds. This finding is important in relation to the observation of breathing in fetuses with prematurely ruptured membranes. The risk of pulmonary hypoplasia is greatest where the membranes rupture before 26 weeks' gestation and much less when they rupture after this time. Blott *et al*[7] and Blott and Greenough[8] suggested that observation of breathing in the human fetus could be used to predict the development of pulmonary hypoplasia in fetuses at risk, but breathing movements are significantly less likely to be seen in normal healthy fetuses before 26 weeks' gestation thus raising questions about the specificity of this test.

Nijhuis *et al*[20] and van Vliet *et al*[21] were the first to study the association between behavioural states and breathing in human fetuses. They reported significantly more episodes of breathing during periods of activity than of quiescence between 32 weeks' gestation and full term, and that the linkage of breathing and other behavioural state variables did not alter with the emergence of behavioural states. Our data are in agreement with both these findings, but in addition show that the association between breathing and activity is present well before 32 weeks' gestation, though this association is not found consistently in all fetuses.

Experimental data on fetal lambs have shown that transition from the rapid eye movement to the non-rapid eye movement state is associated with a 30% reduction in cerebral oxygen and glucose metabolism.[22] Thus in the short term the alteration in behavioural state may provide an important protective response to hypoxic or other stresses. It is possible therefore that the suppression of fetal breathing associated with fetal infection and preterm labour may be secondary to a change in state rather than primary suppression.

The main clinical implications of these data are, firstly, that they emphasise the need for prolonged and repeated recording of breathing activity before any sinister conclusions can be drawn. Secondly, during the last trimester fetal breathing is cycle or state dependent (being significantly less likely during episodes of quiescence) so that studies of the influence of complications of pregnancy on fetal breathing need also to take account of other variables of behaviour. Thirdly, in contrast to fetal breathing, hiccups predominate in immature fetuses and as yet there is no evidence to support their being a useful indicator of fetal health.

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CURRENT TOPIC

Bronchopulmonary dysplasia: then and now

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Bronchopulmonary dysplasia was first described in 1967 in a series of 32 infants with severe respiratory distress syndrome who were treated with artificial ventilation and supplemental oxygen.[1] These infants had initially been given antibiotics, the usual measures for control of body temperature, and glucose and sodium bicarbonate intravenously to combat acidosis. They had not responded to this treatment, were cyanotic in 100% oxygen, looked moribund, and had had one or more prolonged apnoeic spells. The clinical and radiological progression of respiratory distress syndrome was significantly modified by treatment with prolonged positive pressure ventilation, and 80–100% concentrations of oxygen. What was remarkable was the appearance of a new syndrome of chronic lung disease that was called bronchopulmonary dysplasia. The usual course of respiratory distress syndrome before the use of prolonged positive pressure ventilation was that infants who survived the first 3 days of life recovered completely and by 7–10 days of life had normal lungs radiographically.[2] Those who died did so within four days. Chronic lung disease had not been seen as a sequel to respiratory distress syndrome before the description of bronchopulmonary dysplasia.

The clinical, radiological, and pathological progression of bronchopulmonary dysplasia was initially divided into four stages.[1] Stage I (days 1–3) consisted of the initial clinical and radiological picture of respiratory distress syndrome. During stage II (days 4–10) there was increasing opacification of the lungs and, if the infant died, there was thick exudation into the lumen of the airway with patchy bronchiolar and alveolar epithelial necrosis with early stages of repair. At the end of stage II, weaning of the infants from the high concentrations of oxygen (80–100%) could usually be started. Stage III (days 10–20) was a period of transition to chronic lung disease with the chest radiograph showing a reticular network of small rounded areas of radiolucency that represented areas of emphysematous alveoli adjacent to atelectatic alveoli, and pulmonary fibrosis. This stage could be complicated by patent ductus arteriosus and congestive heart failure. In stage IV (after 30 days of age) there was persistent chronic lung disease with hyperexpansion and cystic looking lungs. The electrocardiogram at this stage usually showed right ventricular hypertrophy and cardiomegaly with cor pulmonale. Pathologically, there was emphysema with thickening of the lung interstitium, replacement of type I pulmonary epithelial cells with type II pneumocytes, squamous metaplasia and ulceration of bronchiolar epithelium, hypertrophy of bronchiolar smooth muscle, and early vascular lesions indicating pulmonary hypertension with periarteriolar thickening.

Since the original description of bronchopulmonary dysplasia, it has been described all over the world in places where artificial ventilation and supplemental oxygen have been used to treat infants with severe respiratory distress syndrome. Bronchopulmonary dysplasia has become the most common form of chronic lung disease in infancy in the United States, with about 7000 new cases occurring each year (35 000 cases of respiratory distress syndrome/year times a 0·20 incidence of bronchopulmonary dysplasia/per case of respiratory distress syndrome).[3]

Pathogenesis

The principal factors in the the pathogenesis of bronchopulmonary dysplasia are (i) premature birth, (ii) respiratory failure, (iii) oxygen toxicity, and (iv) barotrauma. Premature birth is associated with immature lung structure, lack of surfactant, and inadequate respiratory drive, all of which contribute to respiratory failure. Respiratory failure is critical in the pathogenesis because it requires treatment with supplemental oxygen and artificial ventilation. The respiratory failure may be from any cause, but is most commonly from respiratory distress syndrome. The interaction of the concentration and duration of supplemental oxygen and the immaturity of the lung determines in part the development of bronchopulmonary dysplasia.[4] Since 1967 the supplemental oxygen concentration used in treating respiratory distress syndrome has been reduced, and many of the infants are less mature. The premature infant who requires supplemental oxygen is at risk of oxygen injury to the lung because the immature antioxidant activity, both enzymatic and non-enzymatic, may not be sufficient to protect against the increased generation of oxygen radicals.[5] The effects of prolonged exposure to concentrations of supplemental oxygen of less than 60% on the immature human or animal lung are not known. Positive pressure ventilation is important in the pathogenesis of bronchopulmonary dysplasia because it facilitates the delivery of supplemental oxygen and can further injure the lung by barotrauma. Pulmonary interstitial emphysema and pneumothorax are the most obvious clinical findings of barotrauma, but damage to the small airways in the surfactant deficient lung can occur without actual rupture of the wall of the airway.[6] Factors that prolong artificial ventilation and supplemental oxygen therapy contribute to the degree of severity of bronchopulmonary dysplasia and include pulmonary air leak (interstitial emphysema, pneumomediastinum, and pneumothorax), pulmonary oedema (patent ductus arteriosus, cor pulmonale, congestive heart failure, and fluid overload), and pulmonary infection. *Ureoplasma urealyticum* has recently been isolated more often from prematurely born infants who developed bronchopulmonary dysplasia than those who did not, but respiratory distress at birth was not associated with colonisation with the organism.[7] The part played by *U urealyticum* in the pathogenesis of bronchopulmonary dysplasia remains to be determined.

Pathology

The changes in the bronchiolar regions in bronchopulmonary dysplasia have been attributed both to barotrauma and to pulmonary oxygen toxicity. Overdistension of airways and irregular aeration of the alveoli can produce sheer forces in the airway mucosa that lead to disruption of the epithelium during artificial ventilation.[8] Necrotising bronchiolitis and squamous metaplasia similar to those seen in bronchopulmonary dysplasia are seen with experimental pulmonary toxicity in newborn mice.[9] The inflammatory cells that accumulate about the small airways and in the alveolar walls amplify the structural damage and enhance the recruitment and proliferation of fibroblasts, which leads to accumulation of type I collagen in these areas.[10] Derangement of pulmonary tissue with almost complete loss of the lung's normal architecture associated with extensive interstitial and interalveolar fibrosis has consistently been described in pathological studies of bronchopulmonary dysplasia.[11–13] Increased numbers of pulmonary inflammatory cells have been well documented in serial bronchoalveolar lavage fluid samples from newborn infants with respiratory distress syndrome who subsequently developed bronchopulmonary dysplasia.[14 15]

Associated with the large numbers of neutrophils in these infants are sustained high elastase, and low α_1-protease inhibitor, activities. This elastase-protease inhibitor imbalance promotes hydrolysis of the connective tissue matrix. As the inflammatory process progresses, alveolar macrophages become more prominent in bronchoalveolar lavage fluid. They also produce an elastase and, when injured, can accelerate the release of neutrophil elastase.[16] Proteolytic enzymes released by inflammatory cells may play an important part in the damage of epithelial and connective tissue in bronchopulmonary dysplasia. Preterm infants may be more susceptible to injury by the proteolytic enzymes because they have low concentrations of serum proteins, including the antiproteases.[17] Because these proteolytic enzymes and the byproducts of the injuries that they cause appear in bronchoalveolar lavage fluid before there is clinical and radiological evidence of bronchopulmonary dysplasia, they may prove useful as early indicators of development of the disease.

Animal experiments

A prematurely delivered immature baboon model for bronchopulmonary dysplasia has been developed.[18] The prematurely delivered baboon develops a syndrome similar to respiratory distress syndrome, and requires artificial ventilation with supplemental oxygen to survive. Treatment with prolonged artificial ventilation and oxygen supplementation does result in the production of stage III bronchopulmonary dysplasia in the immature baboon. It has not been possible with this animal model, however, subsequently to eliminate any of the contributing factors to test the hypothesis that any one of them alone can produce a continuum of pathology similar to bronchopulmonary dysplasia. To date the only single factor that has been shown to produce a continuum of pathology similar to bronchopulmonary dysplasia in its acute, subacute, and chronic forms in a newborn mammalian lung is pulmonary oxygen toxicity.[19]

Incidence

A comparison of the incidence of bronchopulmonary dysplasia in infants with severe respiratory distress syndrome at the Stanford University Medical Center from the original report (1962–1965) and from 1989 is presented in the table. Compared with 1962–5, there was a pronounced decrease in bronchopulmonary dysplasia in 1989 among infants who weighed more than 1500 g at birth ($p<0{\cdot}05$). The overall incidence of bronchopulmonary dysplasia in infants with severe respiratory distress syndrome, however, was not significantly decreased in 1989 compared with 1962–5 because of an increased incidence in infants with respiratory distress syndrome who weighed less than 1500 g. Of note is the high incidence of bronchopulmonary dysplasia among premature

No (%) with stage IV bronchopulmonary dysplasia (BPD) among infants with respiratory distress syndrome (RDS) treated with positive pressure ventilation and oxygen for more than 24 hours

	1962–5: birth weight (g)				*1989: birth weight (g)*			
	<1000	*1000–1500*	*>1500*	*Total*	*<1000*	*1000–1500*	*>1500*	*Total*
BPD/RDS (%):								
Survivors	0/0 (0)	1/2 (50)	3/11 (27)	4/13 (31)	22/30 (73)	8/34 (24)	3/47 (6)	33/111 (30)
Non-survivors	0/3 (0)	2/5 (40)	3/11 (27)	5/19 (26)	3/5 (60)	0/2	1/1 (100)	4/8 (50)
Total	0/3 (0)	3/7 (43)	6/22 (27)	9/32 (28)	25/35 (71)	8/36 (22)	4/48 (8)	37/119 (31)

This table is a shortened and updated version of that published in *Respiratory Distress Syndrome* (Kari O Raivio, Niilo Hallman, Kauko Kouvalainen, Ilkka Välimäki, eds) by Academic Press, 1984.

infants with respiratory distress syndrome weighing less than 1000 g in 1989. In 1962–5, infants weighing less than 1000 g were not artificially ventilated, as the techniques were not sufficiently developed to apply them to these very low birthweight (VLBW) infants. The high incidence of bronchopulmonary dysplasia in VLBW premature infants is an increasing problem.

Radiological changes

The progression of radiological change has been modified as stage IV bronchopulmonary dysplasia has become more common in infants weighing 1000 g or less, and as supplemental oxygen concentrations and peak inspiratory pressures and their durations have decreased.[20] The initial radiograph is usually of immature lung or respiratory distress syndrome, but the lungs may not become completely opaque within the first few days of treatment as originally described, but instead remain persistently hazy for several weeks. At 3 or 4 weeks of age the hazy opacification changes into a fine reticular pattern, with or without hyperexpansion. This reticular pattern may first be noted at 30 days of age, but can initially be seen even later. Its initial appearance may be masked by pulmonary oedema, pneumonia, or poor inspiration. The characteristic radiographic progression of bronchopulmonary dysplasia that was originally described still occurs in the more severely ill infants, particularly those whose course is complicated and prolonged by infection, congestive heart failure, fluid overload, or interstitial emphysema.

The incidence of bronchopulmonary dysplasia seems to be considerably higher in infants who develop pulmonary interstitial emphysema.[21] If the interstitial emphysema is diffuse and persistent, the characteristic radiographic appearance of chronic bronchopulmonary dysplasia will be difficult to differentiate from pulmonary interstitial emphysema. If pulmonary interstitial emphysema persists until the infant is 3 weeks of age, the patient invariably also has bronchopulmonary dysplasia.

Diagnosis

As bronchopulmonary dysplasia has evolved and the radiographic picture has been modified, the diagnostic criteria have become more clinical and physiologically oriented. The diagnosis of bronchopulmonary dysplasia (usually meaning stage IV or chronic disease) includes assessment at 3 or 4 weeks of age, a history of artificial ventilation and supplemental oxygen for at least 24 hours, signs of respiratory distress including an abnormal respiratory rate (greater than 40/minute), persistent intercostal retractions, raised carbon dioxide tension (or requirement for supplemental oxygen for longer than 3 or 4 weeks), and persistent abnormalities on chest radiographs. This approach to the diagnosis of bronchopulmonary dysplasia (recommended by Farrell and Palta) relies on signs and symptoms of the infant's respiratory disease rather than on therapeutic decisions by the physician and should be more replicable among neonatal intensive care nurseries.[3]

Outcome

Increased airway resistance, increased airway reactivity, low dynamic compliance, increased functional residual capacity, increased respiratory rate, high arterial carbon dioxide tension, low arterial oxygen tension, severe maldistribution of ventilation, right or left (or both) ventricular hypertrophy, pulmonary hypertension, and systemic hypertension have been shown in surviving 1 to 2 year old infants with bronchopulmonary dysplasia.[22–31] These abnormalities of cardiopulmonary function can improve with age but may not resolve.[25] Airway obstruction, airway hyper-reactivity, abnormal arterial capillary blood gas values, radiological abnormalities, increased lung volume, and electrocardiographic abnormalities have been found in nine children with a history of bronchopulmonary dysplasia at an average age of 8·4 years.[32] Increased lung volumes, airway obstruction, and increased transcutaneous carbon dioxide tension have been documented in a group of 10 children with an average age of 10·4 years who had bronchopulmonary dysplasia.[33] We have recently shown that most of 22 young adults age 14–23 years who had had bronchopulmonary dysplasia, had pulmonary dysfunction consisting of airway obstruction, airway hyper-reactivity, and air trapping when compared with 20 ex-prematurely born young adults of the same age and 48 normal volunteers of the same age.[34]

Injury to small airways in premature infants' lungs has been a prominent feature and a particularly disturbing aspect of the pathology of bronchopulmonary dysplasia.[9] The possible relationship between lower respiratory illness in childhood and chronic airflow obstruction in adulthood was reviewed by Samet *et al.*[35] Injury to the small airways was the common feature of the respiratory illnesses in childhood, such as infection with respiratory syncytial virus, which might be associated with chronic airway obstruction. In a series of 40 prematurely born infants who had survived artificial ventilation for respiratory insufficiency, 70% had one or more episodes of pneumonia or bronchitis during the first 2 years of life.[36] There was a direct relationship between the presence of bronchopulmonary dysplasia and the number of lower respiratory tract infection. A four month prospective study of 30 children less than 2 years old with bronchopulmonary dysplasia showed that 59% developed respiratory syncytial virus infection and 70% of those required admission to hospital.[37] Premature infants who develop bronchopulmonary dysplasia may be predisposed to have an increased incidence of lower respiratory illnesses and to develop obstructive airways disease as adults.

Many infants with bronchopulmonary dyslasia are below the third centile for height and weight during the first 2 years of life,[38] and persistent growth delay may persist throughout early childhood and beyond. Our follow up study of young adults who had had bronchopulmonary dysplasia indicated that, as a group, they were slightly but significantly shorter and lighter than the normal or prematurely born age matched control group.[34]

Neurodevelopmental deficits have also been reported to occur more often in infants and children with bronchopulmonary dysplasia. It is not clear whether these are caused by bronchopulmonary dysplasia or by some perinatal or neonatal event.[38 39] Neurological abnormalities have been reported in 0 to 38% of premature infants with a diagnosis of bronchopulmonary dysplasia.[40] Developmental abnormalities measured by the Bayley Scales Mental Development Index ranged from 14% to 80% in infants with bronchopulmonary dysplasia. Only one study looked at children as old as 8 years of age, and no significant effect of bronchopulmonary dysplasia on neurodevelopmental outcome was found.[41] Late evaluation of growth and development in young adults who had had bronchopulmonary dysplasia deserves further attention.

Treatment

Reducing the incidence of premature births would be the single most effective way of reducing the incidence of bronchopulmonary dysplasia, and this is both a scientific and a socioeconomic problem. Medical complications of pregnancy and fetal factors leading to premature delivery can be reduced, but not eliminated, by advances in medical science. Availability and use of prenatal care is strongly associated with improved outcome of pregnancy.[42] Provision of adequate prenatal care for all pregnant women and reduction of adverse maternal practices requires educational and social political action.

Once an infant has been born prematurely, the prevention of bronchopulmonary dysplasia rests on preventing the development of respiratory distress. The use of exogenous surfactant as a means of preventing or decreasing the severity of respiratory distress syndrome is currently receiving considerable attention. The effectiveness of several surfactant preparations is being investigated, including those derived from calf and pig lungs, those artificially produced, and human surfactant from amniotic fluid. Comparison of different clinical trials is difficult because of differences in prophylaxis and treatment regimens, gestational ages of the subjects, composition of the exogenous surfactants, outcome measures, and insufficient identification of lung maturity.[43]

The results from several controlled clinical trials are equivocal. Surfactant given prophylactically has decreased the incidence and severity of respiratory distress syndrome.[44–48] Many trials have reported reductions in mortality and in the incidence of pulmonary air leak, and improved gas exchange.[43] A consistent significant reduction in the incidence of bronchopulmonary dysplasia has not, however, been shown. Surfactant seems to be a safe compound, although foreign proteins in some exogenous surfactants have the potential for activating the immune system. Sensitisation has not been shown in humans to date. Three follow up studies have been unable to identify any long term adverse effects of surfactant treatment.[49–51] The exogenous surfactant preparations in use in the United States are considered to be under investigation and the most effective dose of surfactant has not been determined.

The natural antioxidant protection of premature infants may well be inadequate. Because oxygen toxicity is believed to play a part in the pathogenesis of bronchopulmonary dysplasia, the lowest concentration of supplemental oxygen that achieves an arterial oxygen tension of 8·0 kPa or greater should be used to treat the respiratory distress. Enhancing antioxidant protection with exogenous superoxide dismutase may offer some protection against developing bronchopulmonary dysplasia, but this has not yet been thoroughly evaluated. Currently it seems reasonable to attempt to maintain 'normal' levels of activity of antioxidant protection in the face of continuing supplemental oxygen challenge. Nutritional factors that contribute to antioxidant protection such a vitamin E, vitamin C, and β-carotene need to be provided as part of the management of any premature infant likely to develop bronchopulmonary dysplasia.

High frequency positive pressure ventilation, high frequency jet ventilation, and high frequency oscillation have been developed to provide artificial ventilation and reduce barotrauma. It is unclear whether any of these techniques offer any advantages over conventional mechanical ventilation in the routine treatment of respiratory failure of preterm infants. Their use does not seem to decrease the incidence of bronchopulmonary dysplasia, and may be associated with undesirable side effects such as increased incidence of grade III or IV intracranial haemorrhage.[52]

Once the infant has developed bronchopulmonary dysplasia, bronchodilators are often used in management. Their use has been based on the clinical observation that many of these infants had bronchospasm, and infants who died of bronchopulmonary dysplasia had hypertrophied peribronchial smooth muscle.[9] Theophylline has been shown to produce appreciable improvements in airway resistance, specific conductance, complicance, and maximal expiratory flow.[53] The developmental variability in theophylline clearance requires that plasma concentrations be routinely monitored and dosages adjusted. Theophylline toxicity is a concern because it has similar side effects to caffeine, and death may result from serum concentrations above 35 μg/ml.[54] β-2 Adrenergic blocking agents have also been used in the management of bronchospasm in infants with bronchopulmonary dysplasia. The use of inhaled isoproterenol has decreased airway resistance and specific conductance and decreased the work of breathing[55 56]; subcutaneous terbutaline, oral metaproterenol, and inhaled salbutamol have produced similar results.[57 58]

Accumulation of fluids may contribute to the narrowing of small airways, altered pulmonary mechanics, and impairment of gas exchange in infants with bronchopulmonary dysplasia.[59] Treatment with diuretics seems to improve lung mechanics consistently and should decrease the work of breathing in infants with bronchopulmonary dysplasia. This effect is seen in both acute and chronic cases. Frusemide and chlorothiazide are the most commonly used diuretics, and their side effects are related to electrolyte loss in the urine and include the formation of renal calculi.

Corticosteroids have been used in the management of bronchopulmonary dysplasia during the acute, subacute, and chronic phases of the disease. Long acting corticosteroids such as dexamethasone may lead to a short term improvement in pulmonary function and facilitate weaning from mechanical ventilation.[59] Its use for as long as 42 days may improve pulmonary and neurodevelopmental outcome in VLBW infants at high risk of bronchopulmonary dysplasia.[60] The mechanism of action may be a decrease in lung oedema and polymorphonuclear infiltration and an increase in surfactant. The most serious short term side effects are increased risk of infection and hypertension, and with the long term treatment there is the potential for an increased incidence of pulmonary air leaks. Before steroids are used routinely in treating bronchopulmonary dysplasia, further data are needed regarding dose, duration of treatment, and the risk:benefit ratio.

Increasing attention is being paid to the role of nutrition of infants with bronchopulmonary dysplasia.[61] Infants with bronchopulmonary dysplasia at 60 days of age have been shown to absorb energy as well as normal control infants, but they have a lower energy intake and a higher energy expenditure, which results in poor weight gain.[62] They may also have alterations in the composition of their body tissues, with a relatively high water content. Malnutrition has deleterious effects on patients with chronic lung disease and close attention to the nutrition of infants with bronchopulmonary dysplasia is important. The effects of diuretics, gastrooesophageal reflux, increased need of energy, and fluid restriction in infants with bronchopulmonary dysplasia make it difficult to provide them with adequate nutrition.

Infants with bronchopulmonary dysplasia require long periods in hospital. There is, however, an increasing tendency to develop programmes for earlier discharge of these infants while they are still dependent on supplemental oxygen.[63] Infants with bronchopulmonary dysplasia are susceptible to sudden infant death late in their hospital course,[64] and after discharge.[65] Before early discharge is sanctioned, there should be careful review of the infant's cardiorespiratory state and management, including the oxygen requirement, weight gain, medication regimen, and feeding plan.[66] Parental attitude plays an important part in the success of such a programme.

Bronchopulmonary dysplasia remains an appreciable problem for neonatologists. The number of children with bronchopulmonary dysplasia seen by paediatricians is increasing. These children may present with reactive airways disease, recurrent pulmonary infections, and resemble patients with asthma. Though bronchopulmonary dysplasia is thought of as a chronic lung disease of infancy, its results may prove to be life long.

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PERSONAL PRACTICES

Bronchopulmonary dysplasia: early diagnosis, prophylaxis, and treatment

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Survival of extremely premature babies has improved considerably over the last decade.[1] Unfortunately, however, many of these infants remain chronically dependent on respiratory support for many weeks or even months and the most severely affected develop bronchopulmonary dysplasia.[2] The incidence of bronchopulmonary dysplasia among ventilated infants varies, depending on gestational age, from 4·2% to as high as 40%,[1 3 4] but increases up to 70% among infants in whom it is necessary to prolong mechanical ventilation beyond two weeks.[5] Such infants have an increased mortality and morbidity even after discharge from neonatal intensive care. Readmission is common during the first two years of life and infants suffer from recurrent respiratory symptoms with persistent lung function abnormalities. Thus it is most important to determine if the course of this disease may be altered and if it is preventable.

Diagnosis

Early diagnosis is desirable as this would facilitate institution of treatments before irreparable damage has taken place in the hope of altering the disease process. Bronchopulmonary dysplasia or chronic lung disease of prematurity is now diagnosed if infants remain dependent on respiratory support beyond a certain age, usually 1 month in association with an abnormal chest radiograph appearance.[3] Unfortunately such criteria do not allow an early diagnosis. Possibly a more fruitful approach in this respect would be to use attainment of a positive finding from an investigation, if this were shown to be specific for the diagnosis of chronic lung disease of prematurity.

(1) CHEST RADIOGRAPH

Bronchopulmonary dysplasia has usually been diagnosed from the chest radiograph appearance. Northway originally described a progression of changes in the radiograph appearance which were divided into four stages. Stage IV, the most severe, appeared beyond 30 days of age and was associated with hyperexpansion and enlargement of cystic areas, particularly in the lower lobes.[2] This appearance was thought then to be typical of persistent chronic lung disease of prematurity, but nowadays many infants who remain dependent on respiratory support after 1 month of age do not have this classical chest radiograph appearance. Frequently the chest radiograph may only demonstrate homogenous or patchy ill defined opacification, this appearance has been designated as type I chronic lung disease of prematurity.[6] Northway's stage IV bronchopulmonary dysplasia was then reclassified as type II chronic lung disease of prematurity.[6] This differentiation into two types of chronic lung disease of prematurity among infants dependent on respiratory support at 1 month of age was useful, as the two groups had very different prognoses, type I was more common and usually resolved whereas type II usually followed pulmonary interstitial emphysema and had a poor prognosis. A logical use of terminology would be to restrict use of bronchopulmonary dysplasia to indicate a poorer prognosis—that is, those infants with type II chronic lung disease of prematurity. Other infants who remain dependent on respiratory support after 1 month of age, but have less severe chest radiograph changes, would be described as suffering from chronic lung disease of prematurity.

The appearance of the chest radiograph may be further categorised by use of scoring systems. At King's College Hospital we have developed such a scoring system, based on the lung volume and the presence and location of cystic and interstitial elements, which has enabled us to predict which infants will have continuing oxygen dependency at a postconceptional age of 36 weeks or the worst lung function abnormalities. Unfortunately, to date, such scoring systems have usually been based on the chest radiograph appearance at 1 month, and their usefulness regarding an early diagnosis has not been tested.

The chest radiograph appearance of Northway's stage I and II bronchopulmonary dysplasia, which classically appear before 1 month of age, unfortunately may be mimicked by severe respiratory distress syndrome, pneumonia, or heart failure and thus do not allow an early diagnosis. Such conditions may resolve, and thus institution of treatments directed at preventing chronic lung disease of prematurity are inappropriate, particularly those associated with side effects. The chest radiograph appearance associated with stage III bronchopulmonary dysplasia may also have an alternative diagnosis, delayed resolution of pulmonary interstitial emphysema. Both stage III bronchopulmonary dysplasia and persistent pulmonary interstitial emphysema, particularly if present in very immature infants, are both likely to progress to chronic oxygen dependency thus differentiating between these diagnoses in an infant older than 2 weeks of age is not important clinically. Thus persistence of cystic elements beyond the second week of life in very preterm infants could be used to define chronic lung disease of prematurity or at least indicate the need to institute preventative treatment or disease limiting strategies.

(2) TRACHEAL ASPIRATES

It has been claimed that cytology of tracheal aspirate may be more useful in facilitating early diagnosis of chronic lung disease of prematurity,[7] as changes in the aspirate cytology are more advanced than the concurrent chest radiograph appearance. Indeed, this asynchrony has been confirmed by comparison of the chest radiograph appearance to changes in lung tissue at necropsy.[8 9] Classification of aspirate cytology, obtained at the time of endotracheal tube toiletry, is based on the most advanced changes seen. Several groups have sought to separate such changes into distinct stages or classes (C Neave, V Masura. Department of pathology, Women and Infants Hospital, Providence, Rhode Island, 1980).[7 10 11] Class I is characterised by epithelial sloughing, evidence of the destructive process once the injury has stopped, this appearance is usually found between days 0 and 4. Then follow changes which may be evidence of a reparative process with regeneration, scarring and remodelling, but residual damage may still be present depending on the severity of the injury. The following cytologies are characteristic of the sequence of changes in the aspirate: (i) class II (days 4–7) regenerative changes, metaplasia, and early repair of epithelial cells; (ii) transitional class II-III (days 6–12) moderate repair mixed with metaplasia and possible nuclear atypia; (iii) class III definite nuclear atypia and metaplastic cells; and (iv) class IV mature squamous metaplasia, nuclear atypia no longer present. The speed of progression through these classes appears to be dependent on the oxygen dose.[8] The exact importance of these differences in cytological appearance remains controversial. It has been suggested that class III is synonymous with bronchopulmonary dysplasia and results from exposure to high inspired oxygen concentrations and has a variable outcome.[10] Class IV is more severe, occurring in infants intubated for longer than 30 days or with prolonged exposure to oxygen, or both, and there is usually residual pulmonary deficit. Classes I, II, and the transitional class II–III are usually associated with restoration of normal pulmonary growth and function.

Interpretation of the cytology of tracheal aspirates, however, may be hindered because of non-representative sampling of exfoliated cells with imperfect cytological interpretation which makes it difficult to distinguish cellular degeneration from atypia. The presence of histiocytes in the aspirates is unhelpful in diagnosis, as they are present in most aspirates after day 3 and foamy histiocytes occur with use of intravenous intralipid or in congestive heart failure. The presence of neutrophils is more useful in differential diagnosis, although 90% of aspirates on day 4 contain neutrophils, the frequency usually declines to 43% on day 7 but both in transitional and class III it increases again to 85% from days 11–15. Infants who subsequently developed bronchopulmonary dysplasia had a peak incidence of neutrophils at about day 7 which only gradually declined over the next month. Unfortunately neutrophils may also be present in the aspirate because of intercurrent pneumonia or necrotising tracheobronchitis.[12]

Lactosylceramide, an accurate marker of destructive lung inflammation, is strikingly raised in tracheal aspirates of infants who progress to bronchopulmonary dysplasia compared with those of controls when studied in the first week of life.[13] Tracheal aspiration has also been used to document persisting inadequacy of the surfactant system[14] and thus indicate infants who will develop bronchopulmonary dysplasia. In such infants the lecithin:sphingomyelin ratio increases only slowly and,[15] in those in whom the condition is ultimately fatal, phosphatidylglycerol appears several months later than in infants who survive. In 50% of such infants no phospholipid could be detected in the tracheal aspirate collected at gestations corresponding to full term. A major drawback of any diagnostic technique based on tracheal aspirates, however, is that it is only possible to obtain representative sequential samples from intubated infants.

(3) LUNG FUNCTION MEASUREMENTS

Lung function measurements are now possible even in very preterm infants receiving intensive care and are useful in providing an acute assessment of treatment.[16 17] It has also been claimed such measurements, performed in the first week of life, may identify premature infants who will develop bronchopulmonary dysplasia. Although no difference in dynamic compliance could be demonstrated between infants who subsequently did or did not develop bronchopulmonary dysplasia, raised pulmonary resistance on day 1[18] and total airways resistance at a mean age of 4·8 days[19] was found in infants who later developed bronchopulmonary dysplasia compared with controls. The latter study,[19] however, included infants of birth weight less than 750 g and both pulmonary and total airway resistance tend to reflect the size of central airways and thus the results may simply reflect the greater immaturity and lower birth weight of the infants with 'bronchopulmonary dysplasia' compared with the controls. There are many other problems in the interpretation of lung function measurements. Acute changes in lung function may be caused by airway inflammation, fluid overload, or concurrent medication, thus a single result may bear no relationship to the ultimate prognosis. Technical problems such as leaks around the endotracheal tube reduce the accuracy of the measurements. Many commercially available systems are very expensive and may calculate various measurements from pressure changes registered by oesophageal balloons, which are frequently inaccurate in sick preterm neonates, particularly those ventilated and at highest risk of bronchopulmonary dysplasia. 'Home made' systems, which avoid such problems by use of single breath techniques or alternative methods, require considerable expertise both to perform and interpret the measurements. Such factors will continue to prevent such lung function measurements becoming routine practice in neonatal intensive care units until their usefulness outside research studies is more fully proved.

Prophylaxis

A confident early diagnosis thus remains unusual, and prophylactic regimes must be assessed in those infants considered to be at high risk.

(1) VITAMIN E AND SUPEROXIDE DISMUTASE

A number of agents have been given in an attempt to reduce oxygen related lung damage. Premature infants in the first 24 hours of life have inadequate concentrations of vitamin E.[20] Vitamin E deficiency enhances the toxic effects of oxygen upon the lung, which in animals may be prevented by vitamin E treatment. Unfortunately, although results of a non-controlled study were compatible with vitamin E reducing the incidence of infants with a chest radiograph appearance compatible with bronchopulmonary dysplasia,[21] this finding has not been confirmed in randomised controlled trials.[22 23] Although a higher dosage regimen may be necessary to produce the desired therapeutic effect, this is not practical. Vitamin E decreases the oxygen dependent intracellular killing ability of neutrophils, resulting in a decreased resistance to infection[24] and infants maintained at pharmacological concentrations of vitamin E have a significantly higher incidence of proved neonatal sepsis and necrotising enterocolitis.[24]

Human neonates are also deficient in superoxide dismutase,[25] and pretreatment of rats with this substance prevents the toxic changes in lung macrophages exposed to hyperoxia[26] and damage to lung cells.[27] A dosage regimen of 0–25 mg/kg every 12 hours is tolerated in preterm infants without side effects.[28 29] In a randomised controlled trial, superoxide dismutase compared with placebo significantly reduced clinical and radiological evidence of bronchopulmonary dysplasia. Infants given superoxide dismutase required less continuous positive airway pressure (CPAP) and hospitalisation compared with untreated controls.[30] Superoxide dismutase was previously purified from bovine liver but is now produced from recombinant DNA techniques, although no toxicity has been reported it also theoretically could affect the bactericidal activity of polymorphonuclear cells.

(2) VITAMIN A

Vitamin A, administered in a randomised placebo controlled trial as 14 intramuscular injections over a 28 day period also resulted in lower incidence of bronchopulmonary dysplasia, and less need for supplemental oxygen, mechanical ventilation, and intensive care compared with controls.[31] Preterm infants are likely to have low concentrations of vitamin A, because of deprived transplacental acquisition and inefficient parental administration because of photodegradation and absorption to the intravenous tubing, but the exact requirement for vitamin A is not precisely known. If the benefit of vitamin A is confirmed and this treatment is to be given routinely, concentrations must be carefully monitored because of the side effects of this substance given in excess, particularly non-specific neurological abnormalities.

(3) SURFACTANT

Few surfactant studies have addressed the particular question as to whether surfactant treatment reduced bronchopulmonary dysplasia; those that have have yielded promising results. Human amniotic fluid surfactant given as rescue treatment to babies of between 24–29 weeks' gestational age was associated with a significant reduction in bronchopulmonary dysplasia.[32] Larger doses of surfactant seem to be more useful, as a dosage regimen which incorporated doses of 120 mg rather than 60 mg of reconstituted bovine surfactant (surfactant TA) was more effective in preventing both bronchopulmonary dysplasia and intraventricular haemorrhage, although both doses had similar acute effects on improvements in oxygenation.[33] To date no similar effects using calf lung surfactant or artificial lung expanding compound (ALEC) have been demonstrated.

(4) RESPIRATORY SUPPORT

Many authors have incriminated mechanical ventilation as a cause of bronchopulmonary dysplasia. An important method of prevention therefore would be to institute the least damaging form of ventilation. Unfortunately there have been very few studies which in a randomised fashion have compared the effectiveness of two forms of respiratory support with regard to lowering the incidence of bronchopulmonary dysplasia. One such study has recently been carried out, a multicentre randomised controlled trial tested the hypothesis that high frequency oscillation would be associated with a lower incidence of bronchopulmonary dysplasia.[34] High frequency oscillation can provide a method of oxygenation at lower pressures than conventional ventilation in certain infants with severe respiratory failure and thus may be less damaging to the lungs. Unfortunately both conventional ventilation and high frequency oscillation were associated with similar high incidences of bronchopulmonary dysplasia, 40% and 41% respectively and worryingly high frequency oscillation increased the incidence of grade III and IV intraventricular haemorrhage and periventricular leucomalacia.[34]

Other forms of ventilation, patient triggered ventilation[35] and continuous negative extrathoracic pressure (CNEP), have been used anecdotally with apparently good effect in respiratory distress syndrome but no randomised trials have been carried out to assess their role in reducing the incidence of bronchopulmonary dysplasia. It has been suggested early use of nasal CPAP and restricting the use of mechanical ventilation till relatively late in the course respiratory distress syndrome may be associated with a lower incidence of bronchopulmonary dysplasia.[36]

Unfortunately these data come from comparison of eight different centres, which were not randomised to different forms of respiratory support, and variations in the patient population or other management policies may have been responsible for the lower incidence.

A series of studies has demonstrated that ventilation at rates equal to or in excess of 60 breaths/minute, compared with slow rates, is associated with a reduction in pneumothorax which, like bronchopulmonary dysplasia, is related to barotrauma.[37–39] Unfortunately no randomised trial has investigated whether fast rates will also reduce the incidence of bronchopulmonary dysplasia. One study apparently demonstrated that babies ventilated at fast frequencies had significantly worse lung function at follow up compared with those ventilated at conventional frequencies.[40] They further suggested that the degree of lung impairment was related to the length of time spent at the faster rate. The study, however, had a number of impairments; only 14 infants were included, ventilator rates varied from 30–180/minute, and the ventilators used are known to malfunction at fast rates. Using an appropriate ventilator,[41] fast rates, by mimicking the infant's respiratory pattern will induce synchrony[42] improving oxygenation and reduce carbon dioxide concentrations.[43] As a consequence it is possible to reduce mean airway pressure at fast rates and it seems likely that this would be associated with a lower incidence of chronic lung disease of prematurity. At King's College Hospital our routine policy, if infants require ventilation beyond resuscitation, is to commence in the labour suite ventilator rates of 60 breaths/minute. If respiratory efforts remain obvious then the rate is increased till they disappear and the infant is then presumed to be synchronous.[44] If, despite rate manipulation, respiratory efforts remain obvious, the infant is then paralysed and rate reduced to 40–60/minute to avoid airtrapping.[45] Using such a strategy among ventilated very low birthweight infants the incidence of chronic lung disease of prematurity varies each year at King's College Hospital from 15%–20%, this is despite ventilating up to 30 infants per year with birth weights less than 750 g.

Management

(1) WEANING FROM MECHANICAL VENTILATION

As respiratory support has been incriminated as an aetiological factor in the development of bronchopulmonary dysplasia, it is essential not only to reduce peak pressures and the inspired oxygen concentration to the therapeutic minimum, but also to wean infants from respiratory support as soon as possible. A variety of strategies have been tried. Limitation of inspiratory time to a maximum of 0·5 seconds throughout rate reduction is associated with a significantly shortened duration of weaning.[46] Treatment with theophylline reduces the frequency and duration of respiratory pauses which occur in association with bronchopulmonary dysplasia, it improves diaphragmatic function, and reduces pulmonary artery pressure. Benefits regarding lung function which would facilitate weaning, however, have been demonstrated only in the first month of life. Theophylline has a synergistic action on lung function with diuretics[47] and can reduce the duration of weaning.[16 48] Very few extremely preterm infants have been included in such studies and one demonstrated theophylline to be ineffective in ventilated infants older than 30 days of age, as extensive pulmonary fibrosis had resulted in irreversible airways obstruction.[16] In older infants and children long term treatment with theophylline can be associated with multiple side effects including gastrointestinal upset and behavioural disturbance. In preterm infants tachycardia, vomiting, and gastro-oesophageal reflux are increased, as a consequence theophylline concentrations require careful and regular monitoring. This treatment should be restricted to infants in whom ventilator rate reduction has already commenced and thus are likely to wean from ventilation over a few days.

Steroid treatment is also associated with a number of actions which would be potentially beneficial in chronic lung disease of prematurity: reduction of pulmonary oedema, enhancement of surfactant synthesis, reduction of inflammation in injured small airways, and reduction of bronchospasm. A series of uncontrolled trials have indeed demonstrated that administration of steroids improved pulmonary function.[49–51] In one randomised controlled trial improvements in respiratory rate, reduction in peak pressure, and inspired oxygen concentration were reported after a five day course. Two multicentre trials have investigated the impact of this treatment on weaning. The first reported an increase in weaning from respiratory support in ventilator dependent infants at 2 to 6 weeks of age in association with a 64% improvement in pulmonary compliance compared with only a 5% improvement in the placebo group.[52] Unfortunately no significant difference between the two groups was found in mortality or hospital stay, and as a sequential analysis was used only seven pairs of infants were recruited into the study. The second trial recently reported to the British Association of Perinatal Medicine, involved much larger numbers of infants, and confirmed that steroids hastened weaning from ventilator support but was not apparently associated with any benefits in long term outcome. Recent evidence suggests the relatively poor success of steroid treatment in previous trials is explained by the short duration of administration.[53] Weaning from both ventilation and supplemental oxygen and in addition neurological outcome is improved in infants given a 42 day course of steroids compared not only with controls but also with those given an 18 day course of steroids.[53]

Steroid treatment, unfortunately, is associated with a number of side effects that may deter use of this treatment in infants with relatively mild chronic lung disease of prematurity. An increased incidence of pneumothorax, sepsis, necrotising enterocolitis, hyperglycaemia, and hypertension have been reported. The effect on immune function, however, is controversial[54 55]; a recent comparison of infants given a three week course of dexamethasone to controls found no increased incidence of sepsis.[56] A potentially worrying complication, periventricular leucomalacia, has only been reported in one small series.[57] The high incidence of side effects, and at present the limited success of steroids, suggests that this form of treatment should be restricted to ventilator dependent infants in whom no progress with weaning is being made. It is essential to exclude sepsis before commencing treatment and throughout treatment blood pressure must be carefully monitored. There is no evidence that repeated courses of steroids are successful and such a policy is likely to increase the risk of side effects. In infants at highest risk of ventilator damage, that is those of extreme prematurity, if no progress is being made in reduction of ventilator settings at two weeks, it seems reasonable to embark on a prolonged course of steroid treatment providing this is in association with careful monitoring for side effects. An appropriate dosage regimen consists of dexamethasone at 0·5 mg/kg/day for three days, followed by 0·3 mg/kg/day for a further three days, and then 0·1 mg/kg/day for at least two weeks. The precise length the lowest steroid dosage should be maintained remains uncertain, but we have certainly seen acute electrolyte disturbance if the course is terminated too abruptly.

There is a small group of very preterm infants who, despite improvements in their respiratory status, almost immediately require reventilation after extubation with the development of a mixed respiratory and metabolic acidosis. Often no obvious cause is found, but the infants are usually very immature with poor respiratory effort and relatively low muscle mass. After the second such episode we electively continue ventilation at a low rate until the infant is regularly gaining weight and use achievement of a weight milestone (usually 1·0 kg) as criteria for extubation. It has been suggested that CNEP might provide an alternative form of respiratory support in such babies and has the advantage of not involving intubation. Unfortunately, despite recent improvements to the neck seal designed by the Brompton group, there are still problems encountered during CNEP, particularly in the very small infant: poor temperature control, limited access, and facial oedema. No randomised study has been carried out to substantiate if CNEP in this situation has any long term advantage over intermittent mandatory ventilation.

(2) BRONCHODILATORS

Wheezy infants in the first year of life, who were born at term, apparently infrequently show acute benefit from bronchodilators.[58] The response of preterm infants, however, may be different, and those with chronic lung disease of prematurity who have been mechanically ventilated with high inspired oxygen concentrations have morphologic evidence of peribronchiolar smooth muscle hypertrophy.[59] Administration of inhaled bronchodilators to preterm infants during intensive care has indeed been associated with an improvement in lung function. Inhaled isoprenaline causes a reduction in airways resistance and improvement in conductance when given to infants with chronic lung disease at term.[60] Inhaled salbutamol and ipratropium bromide both resulted in similar improvements in lung function,[61] and terbutaline given subcutaneously reduced wheeze as evidenced by an increase in the inspiratory:expiratory ratio. These studies, however, documented improvements in lung function only over, at maximum, one hour and these improvements were not associated with changes in blood gases. The benefits of maintenance treatment with bronchodilators for infants still receiving intensive care remains to be proved. At follow up, however, regular inhaled terbutaline given via a simple spacer device, a coffee cup, reduces symptoms and improves lung function in preterm infants with recurrent cough and/or wheeze,[62] suggesting maintenance treatment during intensive care may be beneficial. Results from follow up studies have demonstrated that it is important to treat only symptomatic infants rather than those with asymptomatic lung function abnormalities. We have recently documented that, whereas nebulised ipratropium bromide acutely improves lung function among symptomatic preterm infants in the first two years of life, it causes a deterioration in airways resistance among infants who have asymptomatic lung function abnormalities.

Despite alterations in the osmolality and pH of the nebuliser solution and removal of the preservative, nebulised treatment has been reported to still cause an acute deterioration in lung function in certain infants born at term.[63] These episodes may be due to bronchomalacia, which is likely to be more common in the preterm infant. In the presence of bronchomalacia, bronchodilator treatment could acutely cause airway collapse and hence result in a deterioration in lung function. It thus seems prudent to restrict treatment with nebulised bronchodilators during intensive care to preterm infants who are obviously wheezy. Ipratropium bromide and salbutamol appear equally effective in the treatment of such infants, both in the acute phase[61] and at follow up.[62]

(3) DIURETICS

Diuretic treatment is also associated with short term improvements in lung function in ventilator dependent infants. Frusemide causes improvements in lung compliance and airways resistance,[17 64] reduction in ventilator requirements,[65] and transitory (two hours) improvements in blood gases.[17] Similar short term rapid improvements in lung function are produced by intravenous diuretic treatment in chronic non-ventilator dependent infants.[66] Maintenance treatment has also been shown to be useful, improving compliance, resistance, and oxygenation at 1 week of age.[67] Chronic use of frusemide unfortunately, however, is associated with a number of side effects, the most important being hypercalcuria, renal calcification, and nephrolithiasis.

It has been suggested that as many as 48% of infants receiving chronic frusemide treatment over an average of 48 days develop renal calcification. The risk is greatest in the most immature infants, those receiving treatment by the intravenous route and for the longest duration. Although the renal calcification and calculi may initially be asymptomatic, infants on this treatment for longer than two weeks should have regular renal ultrasound examinations to facilitate early diagnosis. Renal calcification usually disappears once frusemide is discontinued, and chlorothiazide is beneficial as it reduces urinary calcium excretion,[68] which is associated with dissolution of the calculi. Frusemide treatment is associated with other complications including secondary hyperparathyroidism, rickets, ototoxicity, metabolic alkalosis, and hypochloraemia. Metabolic alkalosis in adults may result in hypoventilation and hypercarbia, but this can be prevented by provision of extra chloride. Ototoxicity is also theoretically preventable as it is related to the plasma concentration of frusemide; unfortunately, however, aminoglycosides have a synergistic action on this side effect.

The thiazide diuretics are potassium sparing and chlorothiazide in particular reduces calcium excretion and thus may be useful in the treatment of hypertensive infants with chronic lung disease of prematurity.[69] These diuretics are less acutely effective than frusemide as their diuretic action is not evident for three to five days after initiating treatment. Maintenance treatment, however, with hydrochlorothiazide and spironolactone may improve outcome,[70] for although no difference in the number of hospital or ventilator days was demonstrated, total respiratory compliance was increased after four weeks of diuretic treatment compared with controls. Commencement of this combination of diuretics in the first weeks of life was also associated with a significant improvement in survival.

Improved knowledge of the action, benefit, and side effects of the various diuretics in preterm infants allows a treatment policy to be drawn up. Frusemide treatment should be restricted to the acute management of fluid overload, particularly in ventilator dependent infants and its use reviewed on a daily basis. Infants chronically diuretic dependent—for example, those in incipient right heart failure—should be maintained on chlorothiazide and spironolactone administered orally.

(4) INFECTION

Infants with chronic lung disease are frequently colonised with bacteria, particularly pseudomonas, although the 'resident' bacteria varies between neonatal intensive care units. Identification of bacteria on microscopy of tracheal aspirate alone is thus not an indication for antibiotic treatment, but rather deterioration in respiratory state in association with both a change in the nature of secretions from the endotracheal tube and the chest radiograph appearance should be the criteria for commencing treatment. The open door policy of visiting practised on most neonatal units means that infants are exposed to many different types of infection including those due to atypical organisms and viruses. To launch a full range of bacterial and viral investigations on each occasion an infant with chronic lung disease of prematurity has a deterioration in respiratory state is clearly impractical. Failure to identify bacteria in the tracheal aspirate during such a change in the infant's condition should prompt at least a nasopharyngeal aspirate to be sent for immunofluorescence to identify respiratory syncytial virus, as treatment with ribavirin seems useful in infants positive for respiratory syncytial virus. Whether other viral infections are important in causing such episodes or if ribavirin would be useful in treating infants with chronic lung disease of prematurity so infected is not known, but is at present under investigation at King's College Hospital. Frequently, bacterial infection is strongly suspected but remains unproved, under such circumstances limiting the duration of antibiotic treatment to a five day course seems appropriate. Infants may suffer from many such episodes and unless an organism is isolated, alternating the choice of antibiotic or antibiotics may avoid the development of resistant organisms. Infants who do not respond to the usual antibiotic combinations and from whom no bacteria or virus is isolated, may show improvement if a prolonged course of cotrimoxazole and erythromycin is instituted.

The high incidence of chronic lung disease of prematurity, particularly in very preterm infants, highlights this as an area essential for research, not only to improve our acute management of affected infants, but most importantly to develop more effective preventative strategies. The results from surfactant replacement treatment are extremely encouraging but it seems likely, despite this tool, we will always have to provide some method of respiratory support at least for the very immature infants. Thus it is essential, if we are to make a major impact on the incidence of chronic lung disease of prematurity, that further research is performed to facilitate tailoring respiratory support more accurately for the requirements of the very preterm infant.

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Bronchopulmonary dysplasia: a new look at management

D P Southall, M P Samuels

Bronchopulmonary dysplasia is an unacceptable but, at present, seemingly unavoidable complication of neonatal intensive care. There are many theories as to why this disorder arises, including lung immaturity, barotrauma from positive airway pressure ventilation, oxygen toxicity, respiratory infections after intubation, and increased lung fluid. Definitions vary, but based on the physiological definition of oxygen dependency at 28 days of age, Boynton has estimated that in the USA 3100 infants are suffering from chronic lung disease at any given time.[1] Based on similar calculations, there may be up to 730 in the UK. After initial hospital discharge, there is a high rate of readmission (up to 60%) and subsequent death (up to 20%).[2]

Having been referred over 90 infants with severe bronchopulmonary dysplasia over the last three years, we have updated our management of infants both in hospital and at home. This paper aims to survey conventional treatment in combination with the presentation of new ideas, including the maintenance of 'normal' oxygenation and the use of negative pressure ventilation as a non-invasive respiratory support.

Clinical management in hospital

(1) DIFFERENTIAL DIAGNOSIS

Infants undergo the following investigations to exclude additional problems where management may be affected: an echocardiogram (for example, to exclude patent ductus arteriosus or anomalous pulmonary venous drainage), sweat test, nasociliary studies, immunoglobulins (including IgE). In selected cases ventilation-perfusion scans may rarely confirm a localised lung defect.

(2) PREVENTION OF HYPOXAEMIA

Inadequate airway oxygenation, as reflected by arterial or transcutaneous measurements, will result in a greater tendency to develop pulmonary vascular hypertension and cyanotic episodes associated with a right to left arteriovenous intrapulmonary shunt.[3] Such episodes may be induced by crying or handling,[3 4] feeding,[5] and infection.[6] They are potentially dangerous and may result in a pulmonary vascular crisis (a major hypoxaemic episode) or sudden death.[7]

In addition to the acute and episodic development of a right to left intrapulmonary shunt, chronic intrapulmonary shunting from inadequate airway oxygenation may produce hypoxaemia over and above that directly due to alveolar hypoventilation. Such shunting may also lead to interstitial pulmonary oedema, similar to that arising from high altitude exposure[8] and may explain why diuretics are of value in some patients with bronchopulmonary dysplasia (see later). While we may use diuretics for established pulmonary hypertension and right heart failure, we would advocate the prevention of this complication. The presence of an accentuated pulmonary component of the second heart sound, right ventricular hypertrophy on electrocardiography or echocardiography, and cyanosis on crying or feeding indicate that airway oxygenation has been inadequate.

The persistent abnormalities in lung surfactant, which have been demonstrated in infants with bronchopulmonary dysplasia,[9] may also result from underperfusion or hypoxia of the alveoli. Thus an adequate airway oxygen tension and adequate effective pulmonary blood flow (that is, without a shunt) may also be important in maintaining function of the alveolar type I and type II cells.

Inadequate oxygenation may also be one cause of the failure to thrive seen in patients with bronchopulmonary dysplasia. There is evidence that additional inspired oxygen may reverse poor weight gain[10] and that weaning infants from additional inspired oxygen too early such that they sustain prolonged periods with arterial hypoxaemia (arterial oxygen saturation (SaO_2) <95%) may divert energy from growth (J R Groothius; Ross Laboratories Special Conference on Bronchopulmonary Dysplasia, Washington DC, December 1989).

Non-invasive and continuous monitoring of oxygenation would ideally involve both accurate transcutaneous oxygen tension ($TcPO_2$) and SaO_2 measurements. Fanconi has shown that pulse oximeters from different manufacturers have different responses, and that before clinical use in neonates they should be validated against arterial line samples measured using a co-oximeter which compensates for fetal haemoglobin.[11] The Nellcor and Ohmeda pulse oximeters are the models most adequately validated in infants,[11–13] although the Ohmeda is less adequate at detecting hyperoxaemia[14] and may under-read the true SaO_2.[15]

In this age group $TcPO_2$ is far less accurate at detecting airway hypoxia than pulse oximetry, in part because of changes in skin blood flow with increasing age.[16] Moreover, the oxygen dissociation curve can be shifted to the right or left depending on 2,3-diphosphoglycerate and fetal haemoglobin concentrations,[17] thus altering the relationship between arterial oxygen pressure (PaO_2) and SaO_2. Although $TcPO_2$ is useful as a trend measurement (see later), we find pulse oximetry a more accurate measure of

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arterial oxygenation and therefore more useful in the definitive evaluation of airway hypoxia.

Before ensuring adequate oxygenation for any infant it is essential to know what is normal at that age and gestation.[18] Two recent investigations performed in our department are relevant. Overnight (12 hour) tape recordings of beat to beat SaO_2 (using a Nellcor N100 pulse oximeter), breathing movements and electrocardiogram have been performed on healthy full term infants at the following ages: in the first month of life (n=43), at around 6 weeks of age (n=67), at around 3 months of age (n=19), and around 6 months of age (n=18). At 6 weeks of age, the median value for the baseline SaO_2 during regular breathing, a state closely correlated with quiet sleep, was 99·8% (range 97·0 to 100%).

In a similar study on an unselected sample of 66 preterm infants at the time of discharge from special care, the median baseline SaO_2 during regular pattern breathing was 99·4%. Moreover, 63 of the 66 subjects had baseline values above 95%.

Thus most preterm and full term infants manifest baseline SaO_2 values between 97 and 100%. These values should therefore be considered as normal when providing additional inspired oxygen to compensate for airway hypoxia resulting from bronchopulmonary dysplasia. Because of the potential dangers of hyperoxaemia in the infant *before* term, the SaO_2 while on additional inspired oxygen must not be permitted to remain at 100% for any length of time. Allowing for an error of ± 2% on the Nellcor pulse oximeter,[12] we maintain SaO_2 between 95 and 98% in patients with bronchopulmonary dysplasia.

When the inspired oxygen requirement is 40% or less maintenance of an adequate inspired oxygen pressure (PO_2) can be achieved by neonatal nasal cannulae (for example, De Vilbiss, Feltham, Middlesex). More than 40% inspired oxygen is provided using a headbox. However, retention of the nasal cannulae may minimise swings in oxygenation that result from disturbances to the headbox oxygen content. It is also important to be aware that handling and medical or nursing procedures, which result in hypoxaemia,[4] may also interrupt the amount of oxygen supplied to the infant at a time when particularly needed. Constant non-invasive monitoring of oxygen concentrations may help prevent iatrogenic hypoxaemia:[19 20] clinical detection based on cyanosis alone will detect only more severe disturbances. When a patient is demonstrating falls in SaO_2/PO_2 on feeding, we provide extra additional inspired oxygen before and during such events. This may mean temporarily increasing the inspired oxygen level from 40 to perhaps 70 or 80%. Noxious procedures, such as the taking of blood or passage of nasogastric tubes, produce crying and may result in severe hypoxaemia. We always provide additional oxygen before such procedures and, as clinically appropriate, adequate local or systemic analgesia or sedation. If an infant is severely upset by a procedure, return to the previous requirements of additional inspired oxygen may take up to several hours.

An additional way of improving oxygenation involves the use of continuous or intermittent negative pressure ventilation.[21] This is described below.

(3) CARBON DIOXIDE RETENTION

Some patients with bronchopulmonary dysplasia have few problems with carbon dioxide retention, suggesting that most of their disease process has selectively affected alveolar gas exchange. Some patients, however, have predominantly small airway disease, recognised by wheeze or evidence of air trapping. In any patient with bronchopulmonary dysplasia and a high arterial carbon dioxide pressure ($PaCO_2$) in the absence of severe oxygenation problems, one must ensure that upper airway obstruction, most commonly resulting from subglottic stenosis, is not present. Laryngeal injury, resulting from endotracheal intubation, has been reported to occur in up to 55% of intubated infants[22] and may be clinically silent. Diagnosis may be achieved by carefully watching the infant's pattern of breathing when asleep, by listening over the larynx with a stethoscope and by tape recordings of inspiratory waveform pattern (inductance plethysmography), SaO_2 and carbon dioxide pressure (PCO_2) concentrations during sleep.[23] Ultimately, endoscopy may be indicated.

Lower airway obstruction may be partly relieved by corticosteroids, methylxanthines, nebulised β agonists or anticholinergics, and occasionally by diuretics. We have also found continuous negative extrathoracic pressure (CNEP) to be of some value; perhaps by increasing the transthoracic pressure gradient, thereby improving airway patency during expiration and effectively replacing the auto positive end expiratory pressure (PEEP) or intrinsic PEEP caused by small airway closure at end expiration.[24] One promising new treatment is the use of nebulised budesonide, a corticosteroid (Astra Pharmaceuticals). Unlike systemic corticosteroids[25] (A Grant; Ross Laboratories Special Conference on Bronchopulmonary Dysplasia, Washington DC, December 1989) neither CNEP or nebulised budesonide have yet been subjected to randomised controlled trial.

(4) NEGATIVE PRESSURE VENTILATION

We use this treatment if more than 40% inspired oxygen is required to maintain SaO_2 at 95 to 98% when quiet or asleep, if there is evidence of noticeable respiratory distress with failure to thrive, and to help in reducing the deterioration that occurs when some infants are weaned from systemic corticosteroids. It is also valuable during intercurrent respiratory infections, particularly in situations where respiratory failure is worsening and further respiratory support is indicated. Being non-invasive it may be used earlier than one would subject an infant to intubation[21] and without the adverse haemodynamic effects produced by intermittent positive pressure ventilation (IPPV). Although positive airway pressure techniques increase ventilation, they increase pulmonary vascular resistances and reduce cardiac output and effective pulmonary blood flow. Thus the gains from improved ventilation may not be fully reflected by improvements in tissue oxygenation. Negative extrathoracic pressure improves ventilation and pulmonary perfusion. Negative pressure may be continuous (CNEP −6 to −8 cm H_2O) or intermittent (intermittent negative extrathoracic pressure of −35/−6, rate 10-60/minute). The latter is initiated when there is increasing carbon dioxide retention or the infant has become tired. CNEP may be given overnight only; the patient breathing unaided during the daytime.

(5) AVOIDANCE OF ANAEMIA

There are two reasons why anaemia should be avoided. Firstly the adverse effects of anaemia on the delivery of oxygen to tissues may impair optimal tissue growth,[17] particularly in the lung. Secondly anaemia may increase the incidence of cyanotic episodes.[26] Correction of anaemia has been shown to reduce the incidence of hypoxaemic episodes,[26] reduce oxygen consumption,[27] and decrease the incidence of apnoeic pauses and periodic breathing.[28 29] We therefore aim to keep the haemoglobin concentration between 110 and 140 g/l. Regular iron and folic acid supplements are provided until the infant is on an adequate mixed diet. If the haemoglobin falls below 110 g/l a blood transfusion is given.

(6) DIURETICS

Controlled trials have shown that diuretics may improve respiratory function in patients with bronchopulmonary dysplasia.[30–32] We use a combination of frusemide (1 mg/kg/dose two to three times per day) and spironolactone (1–1·5 mg/kg twice daily) if right heart failure is present or if there is radiological evidence of interstitial fluid with a persistent oxygenation defect. As described above, however, it should be possible to prevent pulmonary oedema and right heart failure by maintaining adequate airway oxygenation thus avoiding the need for diuretics. Frusemide may cause hypercalcuria and thus in infants with a high alkaline phosphatase activity or radiological evidence of rickets or nephrocalcinosis, chlorthiazide (10–20 mg/kg twice a day) is preferable.

(7) SYSTEMIC CORTICOSTEROIDS

Controlled trials have indicated earlier extubation with corticosteroids, although the durations of oxygen dependency and hospital stay may not be helped.[33 34] Where there is increasing carbon dioxide retention or inability to wean from IPPV we use a three to five day course of dexamethasone at full dosage (0·25 mg/kg every 12 hours). If there is no initial improvement it is discontinued immediately. When an improvement has occurred it is tailed off over seven to 21 days. In those infants in whom deterioration occurs on stopping corticosteroids, we may use nebulised budesonide (0·5–1·0 mg 12 hourly) or alternate day steroids.

We are cautious in commencing corticosteroids because of their predisposition to and suppression of signs from infection. In addition long term effects of these drugs on the development of the fibrous tissue matrix within the lung warrant further study.[35 36]

(8) NUTRITION

As overall body growth is likely to be accompanied by increased lung development, we aim to optimise energy input and utilisation providing 0·63 MJ/kg/day (150 kcal) by supplementing feeds with additional carbohydrate (for example, Polycose (Abbott), Polycal (Cow and Gate), and Maxijul (Scientific Hospital Supplies)) and fat (for example, Liquigen, Calogen, and Duocal (all Scientific Hospital Supplies)). In the infant with persistent respiratory distress, energy consumption may be reduced by minimising the work of breathing, as for example using negative pressure ventilation.[21]

As a compliant ribcage will impair gas exchange in the lung and result in persistent respiratory distress, we monitor for the development of osteopenia of prematurity.[37] In addition to vitamin D supplements, we may also provide calcium and phosphate. Deficiency of the latter may also contribute to persistent respiratory distress from muscle weakness. The potential effects of diuretics and steroids on calcium metabolism also have to be considered (see above).

(9) INFECTION AND ITS TREATMENT AND PREVENTION

Intercurrent respiratory infection frequently begins as a viral infection. Respiratory syncytial virus may be a particular problem for preterm infants in neonatal units as well as the community,[38] with staff sometimes acting as the transmitter of infections.[39] What may simply be a cold or upper respiratory infection for the average baby can be devastating for a patient with bronchopulmonary dysplasia. It is therefore our policy to provide increased protection for all such patients. Parents and nursing or medical staff with an upper respiratory tract infection should rigidly avoid contact with such patients. In the most susceptible infants reverse barrier nursing may be provided. As soon as an upper respiratory tract infection is suspected, a nasopharyngeal aspirate should be taken for immunofluorescence in order to identify respiratory syncytial virus, parainfluenza, or adenovirus, for which it may be helpful to administer nebulised ribavirin. Adenovirus may be particularly problematic as bronchiolitis obliterans may make chronic lung changes irrepairable.

In the systemically ill infant (that is, with a fever, a leucocytosis, increased C reactive protein, and additional changes on chest radiography) we will administer a minimum five day course of broad spectrum intravenous antibiotics.

There is some evidence that *Ureaplasma urealyticum* infection may increase the likelihood of chronic lung disease in preterm infants.[40] We consider administering a seven day course of erythromycin on an empirical basis in the presence of a fever, with or without respiratory symptoms after blood, endotracheal tube, throat, and urine cultures. In most instances, however, the cause of the fever is likely to be viral in origin and the results from serology may help identify the pathogen.

It is important to be aware of the potential danger of the less common pathogens such as monilia, particularly in the patient on corticosteroids. If there is a conjuctivitis, special swabs for chlamydia are taken and the infection treated with a minimum 10 day course of systemic erythromycin.

Early immunisation against pertussis is extremely important. This is begun at three months after delivery, whether or not the patient is in hospital.

(10) MONITORING

All of our patients with bronchopulmonary dysplasia are continuously monitored using a Nellcor pulse oximeter for assessment of baseline hypoxaemia and daily measurement of additional inspired oxygen requirement (see below).[41] During intercurrent respiratory infections, transcutaneous carbon dioxide pressure $TcPCO_2$) is monitored using a sensor heated to 42°C (Hewlett Packard/Draeger electrode) and changed every 12 hours. If there is any deterioration in $TcPCO_2$ or in oxygenation, capillary gas samples can be taken for an analysis of pH, PCO_2, standard bicarbonate, and base excess.

The measurement of additional oxygen requirement is used to assess respiratory function on a daily basis. In non-intubated patients this procedure involves placing the patient's head in a headbox, switching off the nasal cannula oxygen supply, providing oxygen in the headbox, and placing an oxygen analyser (calibrated in air and 100% oxygen) next to the infant's nose. The level of inspired oxygen required to keep the SaO_2 between 95 and 98% is assessed when the patient is quietly asleep. We expect to see oxygen requirements stable—that is, not changing by more than 0·25 l/min through the nasal cannulae, before discharge home is considered.

Home management

PREPARATION FOR HOME

Before discharge a planning meeting is held to which are invited key workers who have been, or will be, responsible for the care of the baby (see table 1). At this meeting the items listed in table 2 are discussed and channels of communication provided.

Table 1 Key workers invited to discharge planning meeting

Unit/ward nurses	Hospital paediatric staff
Family practitioner	Health visitor
Clinical nurse specialist	Liaison health visitor
Social worker	Parents

Table 2 Items for discussion at discharge planning meeting

(1) Home oxygen supply: concentrators, cylinders
(2) Home PO_2 monitor: instruction
(3) Training in resuscitation
(4) Training in respiratory support—for example, negative extrathoracic pressure
(5) Training in nasogastric tube feeding and changing
(6) Discussion of medications, immunisations
(7) Notification of:
    Fire department
    Electricity department
    British Telecom (priority fault repair service)
    Insurance company (for building/equipment)
(8) Identification of lines of communication for medical/equipment problems
(9) Discharge transport
(10) Respite care
(11) Financial help
(12) Follow up:
    Home visits (health visitor, clinical nurse specialist)
    Outpatient

HOME MONITORING

Preterm infants are at increased risk of sudden, unexpected death (between 1/42 and 1/200).[42–46] Those with bronchopulmonary dysplasia are probably at greater risk, whether in hospital[7] or at home.[47] Although these deaths may not be classified as sudden infant death syndrome, the mechanism being thought by some to be different in infants with chronic lung disease, home monitoring is appropriate in this group of infants.[48] Clinical recognition of hypoxaemia is poor; it may easily be undetected[5] (JR Groothius; Ross Laboratories Special Conference on Bronchopulmonary Dysplasia, Washington DC, 1989), and this may be the precursor for more major life threatening events.[3] For the early detection of major cyanotic/apnoeic events, we use a $TcPO_2$ monitor (Kontron 821S). The sensor for this instrument is heated to 43°C and its site changed every eight hours.[49] Although skin PO_2 measurements do not accurately measure PaO_2, compared with SaO_2 measurements from validated pulse oximetry, they are less prone to movement artefact, produce fewer false alarms and may be used continuously when the infant is discharged home. They are also able to identify falls in baseline skin PO_2 indicative of arterial hypoxaemia, for example in the setting of an intercurrent respiratory infection. In 28 infants with bronchopulmonary dysplasia, seven had falls in baseline PO_2, identified by the monitor, and later proved to be hypoxaemia by a validated pulse oximeter in hospital.[49] Three subsequently needed respiratory support because of progressive respiratory failure. Parents rapidly become aware of changes in baseline PO_2 and respond by arranging medical assessment of the infant's clinical state and confirmation or otherwise of arterial hypoxaemia by pulse oximetry. The monitor is also an important tool for the surveillance of additional inspired oxygen supply at home (see below).

DOMICILIARY OXYGEN TREATMENT

To administer oxygen, a home based and a mobile supply both need to be available. The first is provided by an oxygen concentrator which is prescribable by the family practitioner.[50] These are provided by either De Vilbiss, Omnicare, or Rimer-Alco (depending on area), who will visit the home and install the most appropriate system to suit the parents' living conditions. The cost and convenience of a concentrator makes this much more suitable than large cylinders for long term oxygen supply, even taking electricity costs into account. Cylinders are heavy, run out, have a delivery charge of £23, and ultimately are more expensive. When an infant requires 0·5 l/min, the cost per annum of a concentrator is £1030, compared with £3705–4901 for cylinders, depending on whether one or two deliveries per week are needed. Thus we invariably recommend a concentrator to be installed in the home. Parents may be reimbursed from the Family Practitioner Committee the cost of electricity used as this is metered and read by the concentrator supplier.

Secondly, small portable oxygen cylinders (usually size C or D, BOC), are required for when the baby is away from home, either in the car, out in the pram or at relatives' or friends' houses. They may allow mobility for up to 28 hours (see table 3). After negotiation BOC cylinders can usually be replaced when empty by the hospital pharmacy, but if used frequently this may be impractical. Small pin indexed cylinders are available (£494, SOS, Richmond, Surrey) which allow refilling at home from a single large cylinder. This is ultimately more convenient for parents, but involves a more expensive *initial* outlay for the hospital. Oxygen concentrators and small cylinders in most instances will need to be equipped with low flow meters. These are not prescribable by the family practitioner, so must be provided by the hospital and fitted by a qualified technician. De Vilbiss, however, supply them with their oxygen concentrator (for £150, payable by the hospital) and Omnicare may soon follow. Stocks of low flow meters are not large so one should allow ample time for ordering before discharge home.

We have found it preferable to supply oxygen through nasal cannulae as described earlier. Although lower flows can be used with a pernasal pharyngeal catheter, partial occlusion of a nostril and an increase in nasopharyngeal secretions may be a disadvantage of this technique. For emergency use the parents may also be provided with an Ambu bag and face mask of a size suitable for the baby.

Supervision of home oxygen treatment requires regular visits by a specialist nurse who can monitor the infant's oxygen requirement with a headbox and oxygen analyser (as described above), as well as give advice about the clinical aspects of adequate oxygenation. At the time of the visit the nurse will also measure, using a pulse oximeter, the SaO_2 of the baby while quiet and asleep and compare this with the $TcPO_2$ monitor values. As mentioned above, the SaO_2 of normal babies at home is between 97 and 100% when asleep. The aim of additional inspired oxygen should therefore be to keep values in this range. After allowing for a ±2% error on the pulse oximeter (Nellcor), SaO_2 should thus be kept 97–98% when asleep. As lung disease resolves, an overnight tape recording of SaO_2, breathing movements, and electrocardiography may guide the reduction in inspired oxygen concentrations.[23] Short term measurements of oxygen saturation (for example, for 20 minutes) may inadequately reflect the variations that may occur in such infants (JR Groothius; Ross Laboratories Special Conference on Bronchopulmonary Dysplasia, Washington DC, December 1989).

Table 3 Mobility using a size C oxygen cylinder

Flow rate (l/min)	*Hours of oxygen provided (approx)*
0·1	28
0·2	14
0·3	9
0·4	7
0·5	6
0·6	4·5

Parents are often worried that nasal cannulae may become displaced during their sleep. Use of a $TcPO_2$ monitor provides early warning that this has happened. Parents are also worried about electricity cuts. A new three pin plug (B and R Electrical Products, Harlow) that fits any household socket will alarm if this happens thus allowing parents temporarily to supply oxygen from a cylinder. Because of the high dependency of these infants on electricity we notify the local electricity board before their discharge from hospital.

AVOIDANCE OF INFECTION

All respiratory tract infections are potentially dangerous or lethal to the patient with bronchopulmonary dysplasia. It is therefore essential that, as far as possible, such infections are avoided.[51] We therefore do not advise parents to take their infants to the health visitor clinics, preferring the health visitor to visit the patient. Most 'well-baby' clinics, also accommodate preschool children, many of whom carry respiratory infections, especially in the winter months. It is also important that the health visitor herself does not visit a baby with bronchopulmonary dysplasia if she has a respiratory infection. It is impossible to separate older siblings from infants with bronchopulmonary dysplasia, but if they do contract respiratory infections, it is probably best for them temporarily to minimise their contact and handling of the baby. Parental and sibling contact cannot be prevented, but any visitors to the house should not go near the baby if they are suffering from a respiratory infection. Similarly, visits to waiting rooms at doctors' surgeries are to be avoided because of the risk of cross infection. Once again, we request the general practitioner to visit the patient at home in this situation.

Because of the potential danger of pertussis infection in these infants[52] we ensure that infants and their siblings complete their immunisations as soon as possible. There are few contraindications to pertussis immunisation (see Department of Health guidelines[53]) and yet such an infection could be fatal in a baby with bronchopulmonary dysplasia.

It is probably wise to cover respiratory infections with an oral antibiotic, such as amoxycillin, cefaclor, or erythromycin, particularly if there is a fever. All parents are given instruction in the accurate measurement of axillary temperature and taught how to observe an *increase* in chest wall recession, a useful sign of respiratory distress (C J Morley *et al*, personal communication).

Often the baby will manifest low $TcPO_2$ values during an intercurrent infection or immediately after immunisation. These changes are due to either arterial hypoxaemia or reductions in skin blood flow, reflecting the presence of a non-specific illness. We suggest that whenever low $TcPO_2$ values are encountered, they are checked immediately against a pulse oximeter in order to be sure that the infant does not require additional inspired oxygen and/or admission to hospital. In the meantime parents are advised temporarily to increase the inspired oxygen through the nasal cannulas.

NUTRITION

Sometimes it is necessary for feeds to be partly or wholly given by nasogastric tube at home. After appropriate training many parents become adept at passing a nasogastric tube and most would be able to feed their baby using this method. It may also be necessary for milk to have carbohydrate and/or fat supplements (as described earlier). We continue folic acid, iron, and multivitamin preparations until the infant has been weaned onto a mixed diet.

RESPIRATORY SUPPORT

It has sometimes been possible to discharge the infant while still receiving negative extrathoracic pressure respiratory support and for this to be provided at home. In patients who are dependent on this only during the overnight period, we have found that the risks of remaining in hospital outweigh the difficulties of applying this at home. The application of CNEP by parents can be readily taught, but of course, parents need regular supervision with immediate access to the hospital.

EMOTIONAL SUPPORT OF THE PARENTS AND THE BABY

Infants who have severe chronic lung disease and have had prolonged intensive care will be a continual source of anxiety for both parents. It is important that the information they are given is honest, consistent, and regularly updated. This may be difficult when working on a busy unit. Thus the help of other counsellors and therapists may be particularly valuable in providing support. Lines of communication should always be available, and staff should be prepared to advise on more mundane needs such as financial and organisational problems.

PSYCHOMOTOR DEVELOPMENT

It is important that infants with bronchopulmonary dysplasia are given extra exercises and stimulation. Having had protracted neonatal intensive care, their development will more likely be hindered. Parents can be shown by physiotherapists how to stimulate their child appropriately for optimal development. In addition, the consultation of a speech therapist may help prevent the development of feeding difficulties. Once again it is important that any therapist or member of the Portage scheme who visits the infant at home does not do so if they have a respiratory infection.

TRAINING IN CARDIOPULMONARY RESUSCITATION

Provided that airway oxygenation is adequately maintained, cyanotic episodes should not be a problem in these patients. Nevertheless, *all* parents should be able to provide bag and mask ventilation with 100% oxygen and external cardiac massage. This is taught to our parents by clinical nurse specialists with the help of a Resusci Baby (Laerdal Medical), video, and resuscitation manual (the latter available on request). We ask British Telecom to provide for priority repair in the event of faults developing with the home telephone. We also alert the local casualty department and ambulance station concerning the possibility that such an infant will require emergency attention.

Conclusions

There are many new ways in which one can help the infant with bronchopulmonary dysplasia. Most important of these concern the adequate management of airway oxygenation, the monitoring of skin oxygen levels, the avoidance of infection, and the provision of an adequate diet.

We would like to thank the nurses on our intensive care and children's wards for their help in caring so well for these patients. We are extremely grateful to Ms C Angel, Mrs K Jones, Ms J Noyes, Dr C Poets, Dr J Raine, and Dr A Spencer for their valuable ideas and criticisms and Ms B Cronin and Mrs J Lang for typing the manuscript. Dr Samuels is supported by CP Holdings.

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PERSONAL VIEW

Withdrawal of life support in babies: deceptive signals

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Even an ardent 'pro-life' supporter might agree that when a baby has clearly entered the process of dying, and when continuation of ventilatory support is obviously pointless, then it is preferable to allow nature to take its course.

Others hold the view that 'quality of life' is also a consideration. Though the expression has many shades of meaning, those who favour withdrawing life support on the grounds of 'quality of life' agree that the decision must be jointly made with parents. It is the consultant, the doctor who is responsible for directing the care of the baby (and who therefore ultimately takes the responsibility for initiating life support), who must make this decision with the parents. The need to identify a senior person when making decisions is one reason why all babies in maternity units, whether or not admitted to a neonatal unit, should be under the care of a named consultant.

Though the ethics of withdrawing life support from chronically ill newborn babies have been discussed many times, little attention has been given to the circumstances of a baby's care that might prompt a consultant to consider that course of action. When confronted with sensitive issues, nurses and junior doctors often signal their feelings in subtle ways. Although consultants must be receptive to these messages—and indeed may have already begun to question privately the wisdom of continuing life support—early impressions are liable to be deceptive and charged with personal emotion rather than reflect objective and thoughtful appraisal. If we, with parents, are to act 'in the best interests' of the baby we must understand certain pitfalls before initiating discussion with parents.

The following points overlap to some extent, but they are discussed separately because each has rather different implications.

Despair

The frustration felt by staff during the prolonged care of a baby whose course is punctuated by repeated episodes of deterioration and improvement is well known. Despair is an inconsistent emotion. It varies from one member of staff to the next, and is powerfully influenced by the overall amount of stress in the unit. During prolonged periods of stress, perhaps generated by a large work load, despair among staff often becomes endemic and can lead to a consensus opinion that withdrawal of ventilatory support should be a consideration.

For example, a chronically sick baby might 'collapse' (deteriorate acutely) during mechanical ventilation—perhaps as a result of failure to recognise promptly a blocked or misplaced endotracheal tube. After resuscitation, and when the baby is settled once more on the ventilator, attention might be drawn for the first time to the question of withdrawing ventilatory support. The wisdom of resuscitating the baby is, in hindsight, questioned. It may well be that one or more prolonged periods of circulatory failure will indeed compromise long term outcome, but that is an issue which must be judged on its own merit and as scientifically as possible. What must be avoided is bias about the long term or short term prognosis being engendered by the collapse itself, which is a reversible occurrence. Neither staff despair nor parental despair are proper reasons for withdrawing life support.

Appearance of the baby

Sometimes the ravages of chronic neonatal morbidity are reflected in a baby's appearance. I refer here to features that command attention by virtue of their unpleasant and distasteful impact, which cannot be promptly alleviated, and which call to mind the notion of 'suffering'. For example, it may be impossible to meet the energy needs of certain chronically ill babies, and this leads to the appearance of malnutrition. Superimposed on this may be the green-yellow discolouration of the skin in chronic obstructive liver disease associated with parenteral nutrition, and the marks of trauma from surface electrodes and old intravenous infusion sites. However distasteful a baby's appearance may be to staff and parents alike we should strive to disassociate this from considerations of withdrawing life support.

Parents who do not visit

One stimulus for continuing the care of a chronically sick baby is the presence of parents beside the cot. With few exceptions, parents who are concerned about their chronically sick baby encourage the staff to carry on and this feeling of shared responsibility ('we are all in it together') is important. There may be understandable reasons why parents do not visit, but when the element of sharing is not there the incentive for continuing life support may be weakened earlier than it might otherwise be. Our requirement to act in the best interest of the baby applies whether or not his parents are seen to be concerned.

Biased impression of prognosis

There are three main concerns when considering the withdrawal of ventilatory support: the extent to which the baby is perceived to be suffering; the baby's chances of survival; and the anticipated long term outcome should the baby survive. The notion of 'suffering', which is certainly open to biased impressions, is beyond the scope of this short paper.

Given a moral stance that permits withdrawal of life support, the main factor that prompts caution, in my experience, is uncertainty about the short term and long term outcomes. The perception of a baby's prognosis is open to bias in a number of ways. The features of chronic neonatal morbidity, particularly in preterm babies, reflect a stereotyped array of clinical problems. A recent experience of a baby's death, despite prolonged intensive care, may be erroneously used as a yardstick to judge another baby's chances of survival ('It's pointless, he's just the same as baby X and all our care made no difference'). It may be that a baby does indeed have a poor prognosis, but withdrawal of life support should not be based on comparisons with another baby who recently died.

Another source of bias is the interpretation of abnormalities that are seen on ultrasound brain scans. Imagine two babies, both aged 6 weeks, and both with moderate but non-progressive ventriculomegaly diagnosed by brain scan. Ventriculomegaly in the baby no longer requiring ventilatory support may be judged to carry a reasonably good prognosis. In contrast, the same degree of ventriculomegaly in a baby who is receiving assisted ventilation for chronic lung disease may be used to question the wisdom of continuing life support ('he's already got some cerebral atrophy'). Now it may well be that the baby who is more ill does have cerebral atrophy, which might be a real consideration. If so, this interpretation must be made thoughtfully with due regard to the accuracy of diagnosis and implications for prognosis based on scientific observations. We should avoid the double standard whereby the same structural abnormality is used as a pointer towards later severe morbidity in an ill baby but not in a well baby.

If, with parents, we are to act 'in the best interests' of the baby we must acknowledge that subjectivity is not always easy to avoid. Erecting procedural safeguards, such as hospital ethics committees, is not the answer. The solution, in so far as there is one at all, rests with circumspection, self analysis, and the recognition of deceptive signals before voicing an argument for the withdrawing of life support. From the outset we should ask ourselves:

- Am I being driven by despair?
- Am I being unduly influenced by the external appearance of the baby?
- Would I think differently if the parents had been beside the cot every day?
- Are my predictions about outcome based on current scientific knowledge?

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PERINATAL LESSONS FROM THE PAST

Dr William Harvey (1578–1657): physician, obstetrician, and fetal physiologist

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William Harvey was born in Folkstone, Kent, in 1578 and educated first at the Grammar School in Canterbury and then at Caius College Cambridge (1593–7). In 1599, at the age of 21, he travelled to Padua where he studied for the next three years under such masters as Fabricius ab Aquapendente (Anatomy) and Galileo (Mathematics). After obtaining his doctor's degree with high distinction in 1602 he returned to England where he settled in London and married in 1604. The same year he became a member of the College of Physicians and in 1608 was appointed physician to St Bartholomew's Hospital. In 1615 he was made Lumleian lecturer to the College, a post he retained for life. The following year he gave his first dissertation on the circulation of blood, though his most famous work *On the Motion of the Heart and Blood in Animals* was not published until 1628. Meanwhile Harvey had been appointed physician to King James I (c 1618), and subsequently to Charles I. During the Civil War when the Court withdrew to Oxford, he was made Warden to Merton College (1642). In 1646, when 66 years old, he resigned and withdrew from practice. His wife had died and as there had been no offspring, he went to live with one or other of his brothers. In 1651 the second of his great books, that *On the Generation of Animals* was published together with an essay *On Parturition* which was the first original English work on midwifery. In 1654 Harvey presented the College of Physicians with a new building. The same year he was elected President but gracefully declined the office because of his age and infirmities. He suffered badly from gout. So instead he was appointed Consiliari. He died three years later at the age of 79 and was buried in Hempstead, Essex.

William Harvey in 1627.

So much for a bald outline of Harvey's life. For himself, he was a small person with dark eyes and an honest, cheerful countenance. A modest man, he lived in harmony and friendship with his colleagues. He was also a philosopher who liked nothing better than to withdraw to the attic or to a cavern in the garden in order to meditate. His contributions to medicine were immense. He introduced the experimental and observational approach to solving scientific problems and made tremendous contributions to comparitive anatomy and physiology, including embryology, as well as to clinical medicine, surgery and obstetrics. Sadly most of his work was lost when a mob pillaged his home at the outbreak of the Civil War in 1642 and much of the remainder was consumed when the College of Physicians was burnt in the Great Fire of London (1666). The extent of the loss may be gauged by anyone who takes the trouble to study his two remaining published works. They are truly remarkable when judged against the ignorance of the times and the prevalent reliance on ancient authority. Much space would be required to do justice to all his original observations and contributions. Of course, his dramatic exposition on the circulation, which for many years drew such scorn and abuse on his head, eventually achieved for him imperishable fame. Less well known but of similar importance were his observations on the nutrition of the fetus, on the significance of respiration, and on the perinatal circulation, as the following remarkable extracts reveal[1]:

On the placenta:

'In my opinion, then, the placenta (has) an office analogous to that of the liver and mamma . . . This adhesion, or growing together, first takes place, and the fleshy mass (constituting the bond of union between the "conception" and the uterus) is first produced, when the foetus becomes perfectly formed, and, through want either of different or more abundant nourishment, dispatches the extremities of the umbilical vessels to the uterus, that from hence, (as plants do from the earth by their radicles) it may absorb the nutrient juices . . . In truth, the "secundines" are part of the "conception", and depend upon it, borrowing thence their life and faculty of growth. For, just as in the mesentery, the blood is propelled to the intestines by the branches of the coeliac and mesenteric arteries, and returns thence by means of the veins to the liver and heart, together with the chyle, so in like manner do the umbilical arteries carry the blood to the secundines; which blood, together with the nutrient fluid, is brought back by the veins to the foetus . . . the orifices of the umbilical veins are in no way continuous with the uterine vessels . . . the perfect coalition and union, described by Fabricius, never exist. Were it so, the veins and arteries ought to be continuous; for the vessels which bring the blood from the mother into the uterus and carunculae are arteries, whilst those which pass from the uterus to the foetus are veins, as is readily apparent; for they carry blood from the placenta into the vena cava'.

On the independent life of the fetus:
'In the Caesarean section, also, it is quite clear that the life of the embryo does not immediately depend upon the mother, and that the spirits do not proceed from her; for I have often seen the foetus extracted alive from the uterus when the mother has been dead some hours . . . Besides, in a tedious labour we learn whether the infant is alive or not by the pulsation of the umbilical arteries; and it is certain that these arteries receive their impulse from the heart of the foetus and not of the mother, for the rhythm of the two differs: this can be easily ascertained if one hand is applied to the wrist of the mother and the other to the umbilical cord. Nay, in the Caesarean section, when the embryo is still enveloped in the chorion, I have often found the umbilical arteries pulsating, and the foetus lively, even when the mother was dead and her limbs stiffened. It is not, therefore, true that the "spirits" pass from the mother to the foetus through the arteries; nor is it more so that the umbilical or foetal vessels anastomose with those of the uterus. The foetus has a proper life of its own, and possesses pulsating arteries filled with blood and "spirits", long before the "conception", in which it is formed and dwells, is attached to the uterus; just as it is with the chick in the egg'.

On the fetal circulation:
'In the embryo there is not such a difference between the two ventricles; but as in a double nut, they are nearly equal in all respects, the apex of the right reaching to the apex of the left, so that the heart presents itself as a sort of double-pointed cone. And this is so, because in the foetus, as already said, whilst the blood is not passing through the lungs from the right to the left cavities of the heart, but flowing by the foramen ovale and ductus arteriosus, directly from the vena cava into the aorta, when it is distributed to the whole body, both ventricles have in fact the same office to perform, whence their equality of constitution. It is only when the lungs come to be used and it is requisite that the passages indicated should be blocked up, that the difference in point of strength and other things between the two ventricles begin to be apparent: in the altered circumstances the right has only to throw the blood through the lungs, whilst the left has to impel it through the whole body.

Fabricius gives an elegant description, as well as most beautiful figures, of the umbilical vessles . . . these penetrate the foetus at the umbilicus . . . one vein of great size . . . is inserted into the liver of the foetus, and has a communication both with the vena cava and vena portae. In like manner the arteries . . . pass from the uterus towards the foetus, and, gradually uniting and increasing in size, terminate in two large trunks; these, after penetrating the umbilicus, separate from the veins, and attaching themselves to the lateral surface of the bladder by the intervention of a membrane, proceed downwards on either side and become continuous with the branches of the aorta descending to the thigh'.

On fetal sucking:
'The embryo, therefore seeks for and sucks in nourishment by the mouth; and you will readily believe that he does so if you rip him from his mother's womb and instantly put a finger in his mouth; which Hippocrates thinks he would not seize had he not previously sucked whilst in the womb. For we are accustomed to see young infants trying various motions, making experiments, as it were, approaching everything, moving their limbs, attempting to walk, and uttering sounds, acts all of which when taught by repeated experience, they afterwards come to execute with readiness and precision. But the foetus so soon as it is born, aye, before it is born, will suck; doubtless as it had done in the uterus long before. For I have found by experience that the child delayed in the birth, and before it has cried or breathed, will seize and suck a finger put into its mouth. A new-born infant, indeed, is more expert at sucking than an adult, or than he is himself if he have but lost the habit for a few days. For the infant does not suck by squeezing the nipple with his lips as we should, and by suction in the common acceptation; he rather seems as if he would swallow the nipple, drawing it wholly into his throat, and with the aid of his tongue and palate, and chewing, as it were, he milks his mother with more art and dexterity than an adult could practise. He therefore appears to have learned that by long custom, and before he saw the light, which we know full well he unlearns by a very brief discontinuance'.

On the nature of respiration:
'In the mean time I would propose this question to the learned—How does it happen that the foetus continues in its mother's womb after the seventh month? seeing that when expelled after this epoch, not only does it breathe, but without respiration cannot survive one little hour; whilst, as I before stated, if it remain in utero, it lives in health and vigour more than two months longer without the aid of respiration at all. To

state my meaning more plainly—how is it that if the foetus is expelled with the membranes unbroken, it can survive some hours without risk of suffocation; whilst the same foetus, removed from its membranes, if air has once entered the lungs, cannot afterwards live a moment without it, but dies instantly? Surely this cannot be from want of "cooling", for in difficult labours it often happens that the foetus is retained in the passages many hours without the possibility of breathing, yet is found to be alive; when, however, it is once born and has breathed, if you deprive it of air it dies at once. In like manner children have been removed alive from the uterus by the Caesarean section many hours after the death of the mother; buried as they are within the membranes, they have no need of air; but as soon as they have once breathed, although they be returned immediately within the membranes, they perish if deprived of it. If any one will carefully attend to these circumstances, and consider a little more closely the nature of air, he will, I think, allow that air is given neither for the "cooling" nor the nutrition of animals; for it is an established fact, that if the foetus has once respired, it may be more quickly suffocated than if it had been entirely excluded from the air: it is as if heat were rather enkindled within the foetus than repressed by the influence of the air'.

1 Harvey W. *The works of William Harvey*. Translated from the Latin by R Willis. London: The Sydenham Society, 1847:1–624.

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